UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

TZIVA RAPOPORT-HECHT, individually on behalf of herself and all others similarly situated,	:	Case No.
Plaintiff,	:	
v.	:	**CLASS ACTION COMPLAINT**
SEVENTH GENERATION, INC.	:	**JURY TRIAL DEMANDED**
Defendant.	:	**14 CIV. 9087**
	:	JUDGE KARAS

Plaintiff, individually and on behalf of all others similarly situated, by her attorneys, alleges the following upon information and belief, except for those allegations pertaining to Plaintiff, which are based on personal knowledge:

NATURE OF THE ACTION

1. Plaintiff Tziva Rapoport-Hecht ("Plaintiff") brings this action against Seventh Generation, Inc. ("Defendant") on behalf of herself and a class consisting of all consumers in the United States who purchased any of the following Seventh Generation products at any time during the applicable statute of limitations up to and including the present (the "Class Period"):

a. Natural Laundry Detergent

b. Natural 4X Concentrated Laundry Detergent

c. Ultra Power Plus Natural Laundry Detergent

d. Natural Dish Liquid

e. Ultra Power Plus Natural Dish Liquid

(the "Products")

2. In recent years, consumers have become significantly more aware of and sensitive to the toxicity and impact of household products on their health and the health of their families, as well as the impact of these products on the environment in general. As a result, consumer demand has greatly increased for so-called "green" products that are natural and non-toxic.

3. Defendant Seventh Generation portrays itself as a leader of the green movement, claiming on its website and elsewhere to manufacture "Products That Make a Difference," and that "After 25 years of innovation, we've learned a thing or two from plants: they can clean up our world while being kind to our families, Seventh Generation plant-based products get the job done. Without the iffy stuff." (Exhibits A & B)

4. Defendant manufactures the Products (listed in paragraph 1 above) and distributes them to retailers nationwide for sale to consumers at retail locations such as, *inter alia*, Walgreens, Walmart, Target, Amazon.com, Bed Bath & Beyond, Whole Foods, and other grocery stores, drug stores, and health food stores. Seventh Generation also sells the Products directly to consumers on its own website, www.seventhgeneration.com.

5. Seventh Generation represents the Products to be natural, prominently displaying the word "Natural" on the front of the Products, superimposed over the image of a green leaf. (Exhibit C).

6. Unfortunately for consumers, and notwithstanding Seventh Generation's marketing, the representation that the Products are natural is not true, and is in fact a material misrepresentation and deceptive business practice in violation of New York law and consumer protection statutes nationwide.

7. As detailed herein, the Products are not natural, as they contain the non-natural synthetic preservatives benzisothiazolinone and methylisothiazolinone.

FACTUAL ALLEGATIONS

8. Seeking to profit from consumers' desire to locate and use natural and environmentally sound alternatives to standard laundry detergents and dish liquids, Defendant markets the Products as "Natural" in order to capitalize on the recent surge in the popularity of such products.

9. The Products are sold in a variety of outlets, including, *inter alia*, Walgreens, Walmart, Amazon.com, drugstore.com, Whole Foods, Bed Bath & Beyond, Target, and other grocery, drug, and health food stores. Defendant also sells the Products directly to consumers on its own website, www.seventhgeneration.com.

10. Seventh Generation markets the Products as "Natural," prominently including, without qualification, the word "Natural" in the name of each Product. As shown in Exhibit C, Defendant makes this claim on the front of the packaging, which is also illustrated with a large green leaf, further representing the Products as natural.

11. Additionally, each Product label states "It's natural!" and also claims that the Products are "non-toxic" and "clinically *proven* to be hypoallergenic," except for the "Natural 4X Concentrated Laundry Detergent," which is labeled as "non-toxic" and "clinically *tested* to be hypoallergenic." (Exhibit D)(emphasis added).

12. United States regulatory organizations have clearly delineated between natural ingredients and synthetic ingredients. They have not, however, adopted a formal definition of the term "natural."

13. In 2013, the USDA issued a Draft Guidance Decision Tree for Classification of Materials as Synthetic or Nonsynthetic (Natural). In accordance with this decision tree, a substance is natural—as opposed to synthetic—if: (a) it is manufactured, produced, or extracted from a natural source (i.e. naturally occurring mineral or biological matter); (b)it has not undergone a chemical change (i.e. a process whereby a substance is transformed into one or more other distinct substances) so that it is chemically or structurally different than how it naturally occurs in the source material; or (c) the chemical change was created by a naturally occurring biological process such as composting, fermentation, or enzymatic digestion or by hearing or burning biological matter. (Exhibit E).

14. The term "synthetic" is also defined by federal statute as "a substance that is formulated or manufactured by a chemical process or by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral sources, except that such term shall not apply to substances created by naturally occurring biological processes." 7 U.S.C. § 6502(21).

15. Benzisothiazolinone ("BIT") and Methylisothiazolinone ("MIT") are synthetic granular powders that are used as antimicrobial preservatives in material preservatives (used in indoor food and indoor/outdoor non-food), industrial processes and water systems (indoor non-food), and indoor and outdoor residential uses (non-food uses such as the laundry detergents and dish soaps manufactured by Defendant). (Exhibit F; *see also* http://www.epa.gov/pesticides/reregistration/REDs/benzisothiazolin_red.pdf.).

16. Despite Defendant's representations that the Products are "Natural," all of the Products contain BIT and MIT, which are in fact synthetic preservatives.

17. Accordingly, Defendant's claim that the Products are "Natural" is a false, misleading, and material misrepresentation designed to deceive consumers into purchasing the Products at a premium price.

18. In addition to misleading and deceiving consumers into believing that its Products are natural, Defendant also misleads and deceives consumers with false claims about the safety of its so-called "natural" Products, representing the Products to be "non-toxic" and "hypoallergenic." (Exhibit D).

19. Both MIT and BIT have been associated with skin toxicity, immune system toxicity, and allergic reactions, and have been identified as possible neurotoxins. (*See, e.g.*, http://www.ewg.org/skindeep/ingredient/703935/METHYLISOTHIAZOLINONE; http://www.ewg.org/skindeep/ingredient/716930/BENZISOTHIAZOLINONE/)

20. The Environmental Protection Agency has concluded, based on "acute toxicity studies" that MIT is "moderately to highly acutely toxic in oral, dermal, eye irritation, dermal irritation, and inhalation." (Exhibit F).

21. The United Nations Globally Harmonized System of Classification and Labeling of Chemicals has classified MIT as a "strong allergen." (*See* Exhibit G).

22. In 2013 the American Contact Dermatitis Society named MIT the "Contact Allergen of the Year" because of the preservative's known allergenic properties. (Exhibit G).

23. Furthermore, in a March 2014 report by the European Union's Scientific Committee on Consumer Safety ("SCCS"), the SCCS concluded that "current clinical data . . . demonstrate[s] a rapidly increasing frequency of contact allergy to [MIT]." (Exhibit H, p.14).

24. Three recent studies on the issue indicate that MIT causes contact allergy. (*See* Exhibit G).

25. Scientific studies cited by the European Union SCCS indicate that BIT is a significant irritant and elicits contact dermatitis—an allergic reaction—in human test subjects. (*See* Exhibit I).

26. Because of its allergenic and toxic properties, the use of both MIT and BIT has been restricted in both Japan and Canada. (GoodGuide.com, Methylisothiazolinone Information, http://www.goodguide.com/ingredients/53090-methylisothiazolinone).

27. Defendant has thus made false claims about the safety and content of its Products, describing them as "non-toxic" and "hypoallergenic," despite the fact that the Products contain MIT and BIT, both of which are toxic to human skin, to the human immune system, and cause contact dermatitis—an allergic reaction.

28. Seventh Generation misleads and deceives its consumers by labeling its Products and falsely advertising to the public that the Products are natural, when, in fact, they contain synthetic ingredients. Seventh Generation also misleads and deceives consumers by falsely stating and advertising to the public that the Products are safe and non-toxic.

29. The simple fact that Seventh Generation markets and distinguishes its Products as natural when they are not is sufficiently deceptive consumer-oriented behavior to warrant the relief requested herein. The additional fact that there is significant evidence indicating that the Products' synthetic contents are also hazardous to human health—contradicting Defendant's representation that the Products are "non-toxic" and "hypoallergenic"—only serves to highlight the deception perpetrated by Defendant.

JURISDICTION AND VENUE

30. Jurisdiction is proper pursuant to 28 U.S.C. § 1332(d) (2). Plaintiff is a citizen of the State of New York, and Defendant is a corporation organized and existing under the laws of the State of Vermont, with its principal place of business in Vermont. Upon information and belief, the amount in controversy is in excess of $5,000,000, exclusive of interests and costs.

31. This Court has personal jurisdiction over Defendant because Defendant conducts and transacts business in the State of New York, contracts to supply goods within the State of New York, and supplies goods within the State of New York.

32. Venue is proper because Plaintiff and many Class Members reside in the Southern District of New York, and throughout the State of New York.

PARTIES

Plaintiffs

33. Plaintiff Tziva Rapoport-Hecht is an individual consumer who, at all times material hereto, was a citizen of New York residing in the County of Dutchess. Plaintiff purchased Seventh Generation Products throughout 2014 at the Stop and Shop in Dutchess County and at the Walmart in Kingston, New York.

34. Plaintiff purchased the Seventh Generation Products because she saw the labeling, advertising, and read the packaging, which stated, *inter alia,* that the Products were "Natural," "non-toxic," and "hypoallergenic." Plaintiff relied on Defendant's false, misleading, and deceptive representations that the Products were natural. Had Plaintiff known the truth—that the representations she relied upon in making her purchase were false, misleading, and deceptive—she would not have purchased the Seventh Generation Products.

35. Plaintiff was shocked and disappointed to learn of Seventh Generation's deceptive marketing practices, specifically that Defendant's Products not only contained synthetic ingredients, but that those synthetic ingredients are harmful to her health and the health of her family.

36. The members of the proposed class ("Class Members") consist of men and women who live in the United States and purchased the Seventh Generation Products.

Defendant

37. Defendant Seventh Generation is a corporation organized and existing under the law of the State of Vermont, with its principal place of business at 60 Lake Street in Burlington, Vermont, 05401.

38. Defendant manufactures, markets, and distributes the Products throughout New York and the United States.

SUBSTANTIVE ALLEGATIONS

39. Defendant falsely advertises and misrepresents to its consumers—including Plaintiff and Class Members—that Seventh Generation manufactures and sells its Products with natural ingredients. This material misrepresentation induced Defendant's consumers, including Plaintiff and Class Members, to purchase Defendant's Products. Plaintiff and Class Members relied on Defendant's false and misleading misrepresentations.

40. Defendant's statements are false and its practices are deceptive and misleading because, *inter alia*, the Seventh Generation Products contain synthetic ingredients.

41. Defendant falsely advertises and misrepresents to its consumers, including Plaintiff and Class Members, that its "Natural" Products are safe, "non-toxic," and "hypoallergenic." This material misrepresentation induced Defendant's consumers, including Plaintiff and Class Members, to purchase Seventh Generation Products. Plaintiff and Class Members relied on Defendant's false and misleading misrepresentations in purchasing the Products at a premium price above comparable alternatives that are not represented to be "Natural," "non-toxic," and/or "hypoallergenic."

42. Defendant's Products cost a premium relative to comparable alternatives that are not deceptively marketed as natural. The price of Seventh Generation's "Natural Laundry Detergent" relative to the price of Tide and Arm & Hammer brand laundry detergents on www.Walmart.com are exemplary:

i. Whereas Arm & Hammer detergents cost between $0.06 and $0.10 per fluid ounce, and Tide detergents cost approximately $0.12 per fluid ounce, Seventh Generation's purportedly "Natural" laundry detergent costs approximately $0.20 per fluid ounce.

ii. Thus, at Walmart—a massive nationwide retailer with competitive, market-setting prices—Seventh Generation costs 166.6% more than Tide, and 200-333% more than Arm & Hammer alternatives, neither of which appear to be falsely and deceptively marketed as "Natural," "non-toxic," and/or "hypoallergenic free."

43. Defendant's statements are false and its practices are deceptive and misleading because, *inter alia*, the Seventh Generation Products are demonstrably not safe, not "non-toxic," and are not natural.

CLASS ALLEGATIONS

44. Plaintiff brings this matter on behalf of herself and those similarly situated. As detailed at length in this Complaint, Seventh Generation orchestrated deceptive marketing and labeling practices. Seventh Generation customers were uniformly impacted by and exposed to this misconduct. Accordingly, this Complaint is uniquely situated for class-wide resolution, including injunctive relief.

45. The Class is defined as all consumers in the United States who purchased any of the below Seventh Generation Products at any time during the period within the applicable statute of limitations. Each of the following Products is one which Defendant claims is made with natural ingredients, when, in fact, the Products contain synthetic ingredients. Further, Defendant claims that the Products are non-toxic and hypoallergenic, when, in fact, they contain ingredients that are toxic to human skin and the human immune system, and are known contact allergens:

a. Natural Laundry Detergent

b. Natural 4X Concentrated Laundry Detergent

c. Ultra Power Plus Natural Laundry Detergent

d. Natural Dish Liquid

e. Ultra Power Plus Natural Dish Liquid

46. The Class is properly brought and should be maintained as a class action under Rule 23(a), satisfying the class action prerequisites of numerosity, commonality, typicality, and adequacy because:

47. Numerosity: Class Members are so numerous that joinder of all members is impracticable. Plaintiff believes that there are thousands of consumers who are Class Members described above who have been damaged by Defendant's deceptive and misleading practices.

48. Commonality: The questions of law and fact common to the Class Members which predominate over any questions which may affect individual Class Members include, but are not limited to:

a. Whether Seventh Generation is responsible for the conduct alleged herein which was uniformly directed at all consumers who purchased the Products;

b. Whether Seventh Generation's misconduct set forth in this Complaint demonstrates that Seventh Generation has engaged in unfair, fraudulent, or unlawful business practices with respect to the advertising, marketing, and sale of its Products;

c. Whether Seventh Generation made false and/or misleading statements to the Class and the public concerning the content and safety of its Products.

d. Whether Seventh Generation's false and misleading statements concerning its Products were likely to deceive the public;

e. Whether Plaintiff and the Class are entitled to injunctive relief; and

f. Whether Plaintiff and the Class are entitled to money damages under the same causes of action as the other Class Members.

49. Typicality: Plaintiff is a member of the Class. Plaintiff's claims are typical of the claims of each Class Member, in that every member of the Class was susceptible to the same deceptive, misleading conduct and purchased the Seventh Generation Products. Plaintiff is entitled to relief under the same causes of action as the other Class Members.

50. Adequacy: Plaintiff is an adequate Class representative because her interests do not conflict with the interests of the Class Members she seeks to represent; her consumer fraud claims are common to all members of the Class and she has a strong interest in vindicating her rights; she has retained counsel competent and experienced in complex class action litigation and they intend to vigorously prosecute this action. Plaintiff has no interests which conflict with those of the Class. The Class Members' interests will be fairly and adequately protected by Plaintiff and her counsel. Seventh Generation has acted in a manner generally applicable to the Class, making relief appropriate with respect to Plaintiff and the Class Members. The prosecution of separate actions by individual Class Members would create a risk of inconsistent and varying adjudications.

51. The Class is properly brought and should be maintained as a class action under Rule 23(b) because a class action is superior to traditional litigation of this controversy. Pursuant to Rule 23(b)(3), common issues of law and fact predominate over any other questions affecting only individual members of the Class. The Class issues fully predominate over any individual issue because no inquiry into individual conduct is necessary; all that is required is a narrow focus on Seventh Generation's deceptive and misleading marketing and labeling practices. In addition, this Class is superior to other methods for fair and efficient adjudication of this controversy because, *inter alia*:

52. Superiority: A class action is superior to the other available methods for the fair and efficient adjudication of this controversy because:

a. The joinder of thousands of individual Class Members is impracticable, cumbersome, unduly burdensome, and a waste of judicial and/or litigation resources;

b. The individual claims of the Class Members may be relatively modest compared with the expense of litigating the claim, thereby making it impracticable, unduly burdensome, and expensive—if not totally impossible—to justify individual actions;

c. When Defendant's liability has been adjudicated, all Class Members' claims can be determined by the Court and administered efficiently in a manner far less burdensome and expensive than if it were attempted through filing, discovery, and trial of all individual cases;

d. This class action will promote orderly, efficient, expeditious, and appropriate adjudication and administration of Class claims;

e. Plaintiff knows of no difficulty to be encountered in the management of this action that would preclude its maintenance as a class action;

f. This class action will assure uniformity of decisions among Class Members;

g. The Class is readily definably and prosecution of this action as a class action will eliminate the possibility of repetitious litigation;

h. Class Members' interest in individually controlling the prosecution of separate actions is outweighed by their interest in efficient resolution by single class action; and

i. It would be desirable to concentrate in this single venue the litigation of all plaintiffs who were induced by Defendant's uniform false advertising to purchase its "Natural" Products.

53. Accordingly, this Class is properly brought and should be maintained as a class action under Rule 23(b)(3) because questions of law or fact common to Class Members

predominate over any questions affecting only individual members, and because a class action is superior to other available methods for fairly and efficiently adjudicating this controversy.

INJUNCTIVE CLASS RELIEF

54. Rules 23(b)(1) and (2) contemplate a class action for purposes of seeking class-wide injunctive relief. Here, Seventh Generation has engaged in conduct resulting in misleading consumers about ingredients in its Products. Since Seventh Generation's conduct has been uniformly directed at all consumers in the United States, and the conduct continues presently, injunctive relief on a class-wide basis is a viable and suitable solution to remedy Defendant's continuing misconduct.

55. The injunctive class is properly brought and should be maintained as a class action under Rule 23(a), satisfying the class action prerequisites of numerosity, commonality, typicality, and adequacy because:

a. Numerosity: Individual joinder of the injunctive class Members would be wholly impracticable. Seventh Generation Products have been purchased by thousands of persons in the United States.

b. Commonality: Questions of law and fact are common to members of the Class. Defendant's misconduct was uniformly directed at all consumers. Thus, all members of the Class have a common cause against Seventh Generation to stop its misleading conduct through an injunction. Since the issues presented by this injunctive class deal exclusively with Defendant's misconduct, resolution of these questions would necessarily be common to the

entire Class. Moreover, there are common questions of law and fact inherent in the resolution of the proposed injunctive class, including, *inter alia*:

i. Resolution of the issues presented in the 23(b)(3) class;

ii. Whether members of the Class will continue to suffer harm by virtue of Defendant's deceptive product marketing and labeling; and

iii. Whether, on equitable grounds, Defendant should be prevented from continuing to deceptively mislabel its Products as "Natural."

c. Typicality: Plaintiff's claims are typical of the claims of the injunctive class because her claims arise from the same course of conduct (i.e. Defendant's deceptive and misleading marketing, labeling, and practices). Plaintiff is a typical representative of the Class because, like all members of the injunctive class, she purchased Seventh Generation Products which were sold unfairly and deceptively to consumers within the United States.

d. Adequacy: Plaintiff will fairly and adequately represent and protect the interests of the injunctive class. Her consumer protection claims are common to all members of the injunctive class and she has a strong interest in vindicating her rights. In addition, Plaintiff and the Class are represented by counsel who is competent and experienced in both consumer protection and class action litigation.

56. The injunctive class is properly brought and should be maintained as a class action under Rule 23(b)(2) because Plaintiff seeks injunctive relief on behalf of the Class Members on grounds generally applicable to the entire injunctive class. Certification under Rule 23(b)(2) is appropriate because Seventh Generation has acted or refused to act in a manner that

applies generally to the injunctive class (i.e. Defendant has marketed its Products using the same misleading and deceptive labeling to all of the Class Members). Any final injunctive relief or declaratory relief would benefit the entire injunctive class as Seventh Generation would be prevented from continuing its misleading and deceptive marketing practices and would be required to honestly disclose to consumers the nature of the contents of its Products.

FIRST CAUSE OF ACTION
VIOLATION OF NEW YORK GBL § 349
(On Behalf of Plaintiff and All Class Members)

57. Plaintiff repeats and realleges each and every allegation contained in all the foregoing paragraphs as if fully set forth herein.

58. New York General Business Law Section 349 ("GBL § 349") declares unlawful "[d]eceptive acts or practices in the conduct of any business, trade, or commerce or in the furnishing of any service in this state . . ."

59. The conduct of Defendant alleged herein constitutes recurring, "unlawful" deceptive acts and practices in violation of GBL § 349, and as such, Plaintiff and the Class Members seek monetary damages and the entry of preliminary and permanent injunctive relief against Seventh Generation, enjoining it from inaccurately describing, labeling, marketing, and promoting its Products.

60. There is no adequate remedy at law.

61. Defendant misleadingly, inaccurately, and deceptively presents its Products to consumers.

62. Defendant's improper consumer-oriented conduct—including labeling and advertising the Products as "natural," and misrepresenting that its Products are safe and "non-

toxic"—is misleading in a material way in that it, *inter alia*, induced Plaintiff and Class Members to purchase and pay a premium for Defendant's Products and to use those Products when it otherwise would not have.

63. Plaintiff and the Class members have been injured inasmuch as they paid a premium for products that were—contrary to Defendant's representations—not natural, not safe, and not "non-toxic." Accordingly, Plaintiff and the Class Members received less than what they bargained and/or paid for.

64. Defendant's advertising and Product labeling induced the Plaintiff and Class Members to buy Defendant's Products.

65. Defendant's deceptive and misleading practices constitute a deceptive act and practice in the conduct of business in violation of New York General Business Law §349(a) and Plaintiff and the Class have been damaged thereby.

66. As a result of Defendant's recurring "unlawful" deceptive acts and practices, Plaintiff and Class Members are entitled to monetary damages, injunctive relief, restitution, and disgorgement of all monies obtained by means of Defendant's unlawful conduct, interest, and attorney's fees and costs.

SECOND CAUSE OF ACTION
VIOLATION OF NEW YORK GBL § 350
(On Behalf of Plaintiff and All Class Members)

67. Plaintiff repeats and realleges each and every allegation contained in all the foregoing paragraphs as if fully set forth herein.

68. N.Y. Gen. Bus. Law § 350 provides, in part, as follows:

> False advertising in the conduct of any business, trade or commerce or in the furnishing of any service in this state is hereby declared unlawful.

69. N.Y. Gen. Bus. Law § 350a(1) provides, in part, as follows:

> The term 'false advertising, including labeling, of a commodity, or of the kind, character, terms or conditions of any employment opportunity if such advertising is misleading in a material respect. In determining whether any advertising is misleading, there shall be taken into account (among other things) not only representations made by statement, word, design, device, sound or any combination thereof, but also the extent to which the advertising fails to reveal facts material in the light of such representations with respect to the commodity or employment to which the advertising relates under the conditions proscribed in said advertisement, or under such conditions as are customary or usual . . .

70. Defendant's labeling and advertisement contain untrue and materially misleading statements concerning Defendant's Products inasmuch as they misrepresent that the Products contain natural ingredients, and they misrepresent that the Products are safe and "non-toxic."

71. Plaintiff and the Class Members have been injured inasmuch as they relied upon the labeling and advertising and paid a premium for products that were—contrary to Defendant's representations—not natural, not safe, and not "non-toxic." Accordingly, Plaintiff and the Class Members received less than what they bargained and/or paid for.

72. Defendant's advertising and product labeling induced the Plaintiff and Class Members to buy Defendant's Products.

73. Defendant knew, or by exercising reasonable care should have known, that its statements and representations as described in this Complaint were untrue and/or misleading.

74. Defendant's conduct constitutes multiple, separate violations of N.Y. Gen. Bus. Law § 350.

75. Defendant made the material misrepresentations described in this Complaint in Defendant's advertising and on its Products' labels.

76. Defendant's material misrepresentation were substantially uniform in content, presentation, and impact upon consumers at large. Moreover, all consumers purchasing the Products were and continue to be exposed to Defendant's material misrepresentations.

77. As a result of Defendant's false or misleading labeling and advertising, Plaintiff and Class Members are entitled to monetary damages, injunctive relief, restitution, and disgorgement of all monies obtained by means of Defendant's unlawful conduct, interest, and attorneys' fees and costs.

THIRD CAUSE OF ACTION
BREACH OF EXPRESS WARRANTY
(On Behalf of Plaintiff and All Class Members)

78. Plaintiff repeats and realleges each and every allegation contained in the foregoing paragraphs as if fully set forth herein.

79. Defendant provided the Plaintiff and Class Members an express warranty in the form of written affirmations of fact promising and representing that its Products were made with natural ingredients and were safe and non-toxic.

80. The above affirmations of fact were not couched as "belief" or "opinion" and were not "generalized statements of quality not capable of proof or disproof."

81. These affirmations of fact became part of the basis for the bargain and were material to the transaction for the Plaintiff's and Class Members' transactions.

82. Plaintiff and Class Members reasonably relied upon the Defendant's affirmations of fact and justifiably acted in ignorance of the material facts omitted or concealed when they decided to buy Seventh Generation Products.

83. Defendant was given opportunities to cure its default but refused to do so.

84. Contrary to Seventh Generation's affirmations of fact, Defendant breached the express warranty because Seventh Generation Products contain synthetic ingredients, are unsafe, and are not "non-toxic."

FOURTH CAUSE OF ACTION
BREACH OF IMPLIED WARRANTY OF MERCHANTIBILITY
(On Behalf of Plaintiff and All Class Members)

85. Plaintiff repeats and realleges each and every allegation contained in the foregoing paragraphs as if fully set forth herein.

86. Defendant is in the business of manufacturing, producing, distributing, and selling laundry detergents and dish liquids.

87. Under the Uniform Commercial Code's implied warranty of merchantability, the Defendant warranted to the Plaintiff and the Class Members that the Products contain natural ingredients and are safe and "non-toxic."

88. Defendant breached the implied warranty of merchantability in that Seventh Generation's Products' ingredients naturally deviate from the label and product description, and reasonable consumers expecting a product that conforms to its label would not accept the

Seventh Generation Products if they knew that they actually contained synthetic ingredients, are unsafe, and not "non-toxic."

89. Defendant breached the implied warranty of merchantability. Seventh Generation Products contain synthetic ingredients. Furthermore, the Defendant's advertising falsely states that the Products are safe and "non-toxic." Reasonable consumers expecting a product that conforms to its label would not accept the Seventh Generation Products if they knew that the Products contained synthetic ingredients, and are unsafe and not "non-toxic."

90. Defendant breached the implied warranty of merchantability in that the Seventh Generation Products do not conform to the promises or affirmations made on the Products' containers or labels or literature, as Seventh Generation Products contain synthetic ingredients. Furthermore, the Products are not safe and non-toxic. Any reasonable consumer would not accept the Seventh Generation Products if they knew that the Products actually contained synthetic ingredients that are known to be toxic to human skin, the human immune system, and that are known to cause allergic reactions.

91. Within a reasonable amount of time after the Plaintiff discovered that the Products contain synthetic ingredients that are associated with human toxicity, Plaintiff notified the Defendant of such breach.

92. The inability of the Seventh Generation Products to meet the label description was wholly due to the Defendant's fault and without Plaintiff's fault or neglect, and was solely due to the Defendant's manufacture and distribution of the Products to the public.

93. As a result of the foregoing, Plaintiff and the Class Members have been damaged in the amount paid for the Seventh Generation Products, together with interest thereon from the date of purchase.

FIFTH CAUSE OF ACTION
BREACH OF IMPLIED WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE
(On Behalf of Plaintiff and All Class Members)

94. Plaintiff repeats and realleges each and every allegation contained in the foregoing paragraphs as if fully set forth herein.

95. Plaintiff and other Class Members bought the Seventh Generation Products with the specific purpose of buying cleaning products that were safe, non-toxic, and contained exclusively natural ingredients.

96. Defendant knew or had reason to know that the Plaintiff and other Class Members were buying its Products with the specific purpose of buying cleaning products that were safe, non-toxic, and contained exclusively natural ingredients.

97. Plaintiff and the other Class Members, intending to use safe, non-toxic, and wholly natural cleaning products, relied on the Defendant in selecting its Products to fit their specific intended use.

98. Defendant held itself out as having particular knowledge of the Seventh Generation Products' ingredients, safety, and toxicity.

99. Plaintiff and the other Class Members reliance on Defendant in selecting Seventh Generation Products to fit their particular purpose was reasonable given Defendant's claims and representations in its advertising and labels concerning the Products' ingredients, safety, and toxicity.

100. Plaintiff and the other Class Members' reliance on Defendant in selecting Seventh Generation Products to fit their particular use was reasonable given Defendant's particular knowledge of the Products it manufactures and distributes.

101. As a result of the foregoing, Plaintiff and the Class Members have been damaged in the amount paid for the Seventh Generation Products, together with interest thereon from the date of purchase.

SIXTH CAUSE OF ACTION
COMMON LAW UNJUST ENRICHMENT
(On Behalf of Plaintiff and All Class Members)

102. Plaintiff repeats and realleges each and every allegation contained in the foregoing paragraphs as if fully set forth herein.

103. Plaintiff, on behalf of herself and consumers nationwide, brings a common law claim for unjust enrichment.

104. Defendant's conduct violated, *inter alia*, New York General Business Law §§ 349 and 350 by manufacturing, advertising, marketing, and selling its Products while misrepresenting and omitting material facts.

105. Defendant's unlawful conduct as described in this Complaint allowed Defendant to knowingly realize substantial revenues from selling its Products at the expense, and to the detriment or impoverishment, of the Plaintiff and Class Members, and to the Defendant's benefit and enrichment. Defendant has thereby violated fundamental principles of justice, equity, and good conscience.

106. Plaintiff and Class Members conferred significant financial benefits and paid substantial compensation to Defendant for Products that were not as defendant represented them to be.

107. Under New York's common law principles of unjust enrichment, it is inequitable for Defendant to retain the benefits conferred by Plaintiff's and Class Members' overpayments.

108. Plaintiff and Class Members seek disgorgement of all profits resulting from such overpayments and establishment of a constructive trust from which Plaintiff and Class Members may seek restitution.

JURY DEMAND

Plaintiff demands a trial by jury on all issues.

WHEREFORE, Plaintiff, on behalf of herself and the Class, prays for judgment as follows:

(a) Declaring this action to be a proper class action and certifying Plaintiff as the representative of the Class under Rule 23 of the FRCP;

(b) Entering preliminary and permanent injunctive relief against Seventh Generation, directing Seventh Generation to correct its practices and to comply with consumer protection statutes nationwide, including New York consumer protection law;

(c) Awarding monetary damages, including treble damages, pursuant to GBL § 349 and GBL § 350.

(d) Awarding punitive damages.

(e) Awarding Plaintiff and Class Members their costs and expenses incurred in this action, including reasonable allowance of fees for Plaintiff's attorneys and experts, and reimbursement of Plaintiff's expenses; and

(f) Granting such other and further relief as the Court may deem just and proper.

Dated: 11/13/14

THE SULTZER LAW GROUP, P.C.

By: _____

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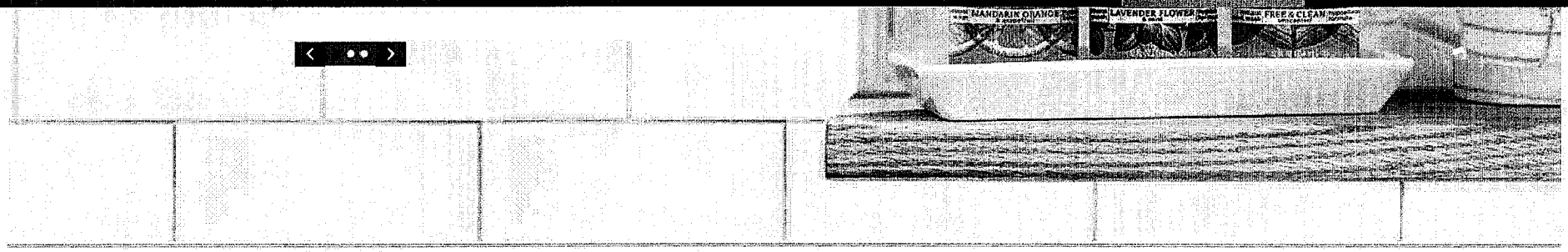
EXHIBIT A

http://www.seventhgeneration.com/

Green Cleaning Products, B...

Suggested Sites | Get more Add-ons

US | Sign up for email savings! | FIND A STORE | CONTACT US | SIGN IN | 0 CHECKOUT



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Up to 25%

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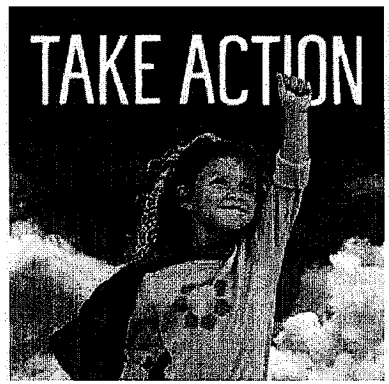
After 25 years of innovation, we've learned a thing or two from plants: they can clean up our world while being kind to our families, Seventh Generation plant-based products get the job done. Without the iffy stuff.

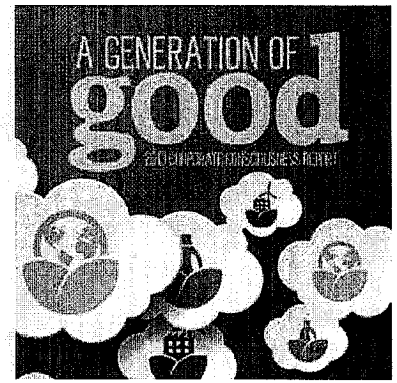
LEARN MORE ABOUT OUR MISSION >

READ OUR BLOG >



Reduce Chemicals of Concern in the Environment

SIGN THE PETITION



2013 Corporate Consciousness Report

VIEW NOW

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11/3/2014

EXHIBIT B

http://www.seventhgeneration.com/mission

Our Mission | Seventh Gene... ×

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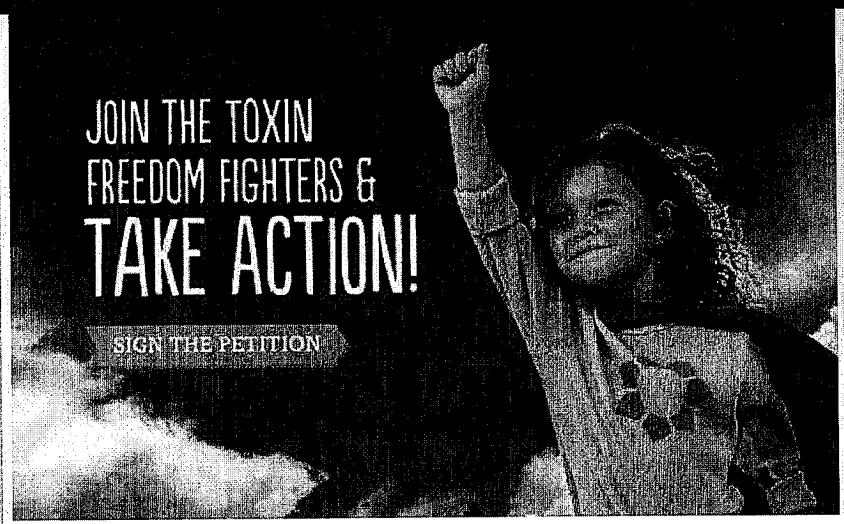

To Inspire
a consumer revolution that **nurtures the health** of the next seven generations.

Our 2020 goals push us to bring sustainable and innovative products to market. **SEE OUR GOALS›**

We Made A Promise The Moment We Named Our Brand

From our very first days as a company we have been driven to consider the effects of our actions on the well-being of the next seven generations.

Products That Make a Difference

We believe with each new product we make, we must also build new ways of caring for ourselves, and the world around us. We can use the power of business to start a movement that will

OUR VISION

gen

Explore our 2013 Sustainability Report

CLICK HERE

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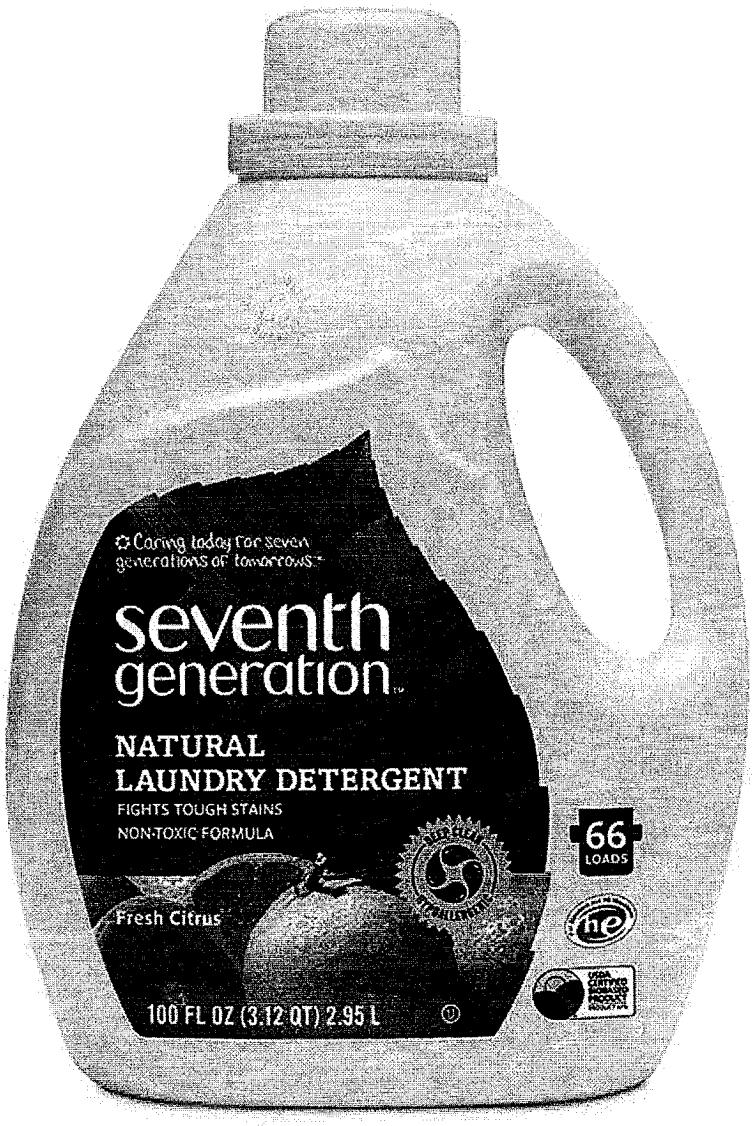
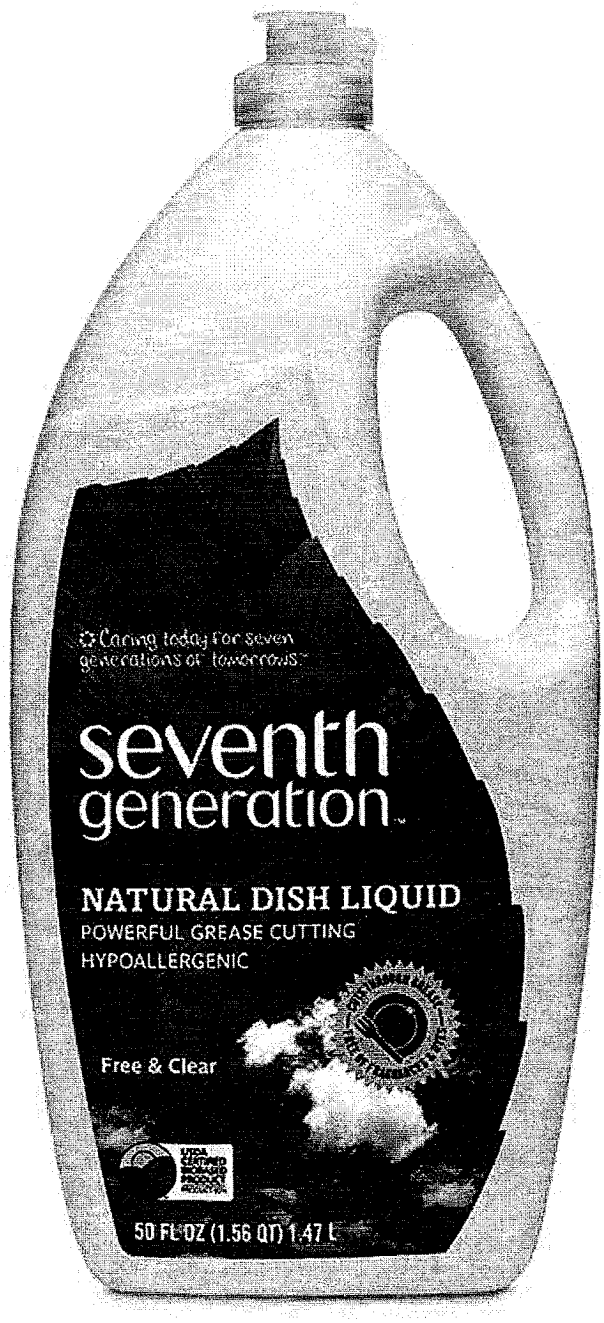
EXHIBIT C


Caring today for seven generations of tomorrows
seventh generation
NATURAL
LAUNDRY DETERGENT
FIGHTS TOUGH STAINS
NON-TOXIC FORMULA
Fresh Citrus
66 LOADS
100 FL OZ (3.12 QT) 2.95 L


53 LOADS
SMALL BOTTLE BIG CLEAN
seventh generation
4X ULTRA CONCENTRATED
NATURAL LAUNDRY DETERGENT
DEEP CLEAN • GENTLE ON SKIN • NON-TOXIC FORMULA
Free & Clear
40 FL OZ (1.25 QT) 1.18 L
53 LOADS
53 LOADS
53 LOADS
(1.25 QT) 1.18 L


NEW
seventh generation
ULTRA POWER-PLUS
WITH ADDED PlantTech STAIN-LIFTING ENZYMES
FRESH SCENT
64 LOADS
he
with PlantTech
25% MORE
STAIN FIGHTING POWER
96 FL OZ (2.96 QT) 2.8 L


Caring today for seven generations of tomorrows
seventh generation
NATURAL DISH LIQUID
POWERFUL GREASE CUTTING
HYPOALLERGENIC
Free & Clear
50 FL OZ (1.56 QT) 1.47 L

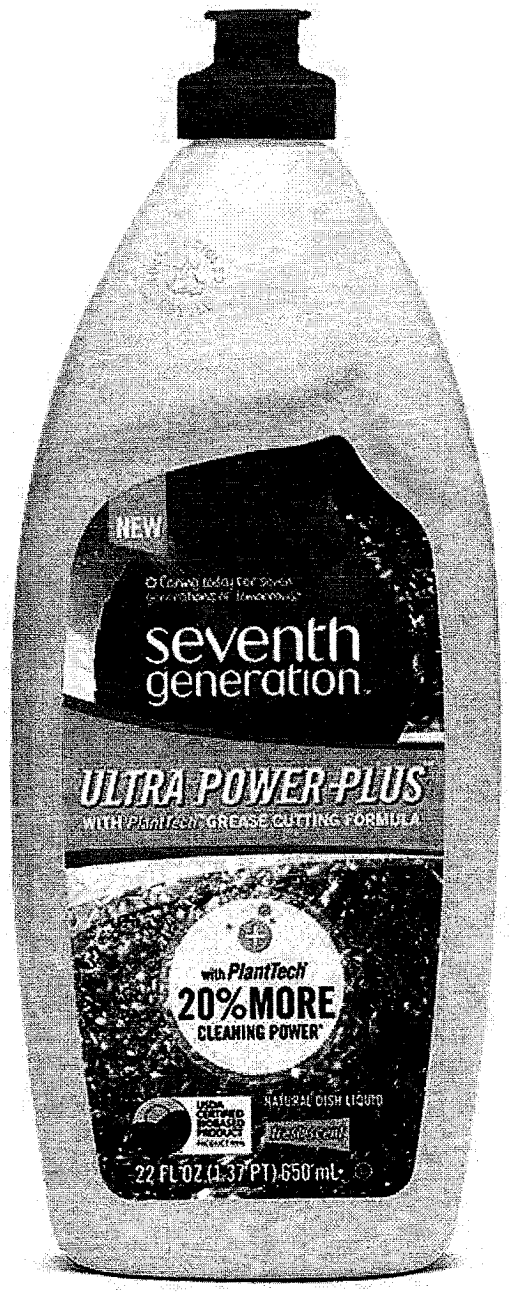
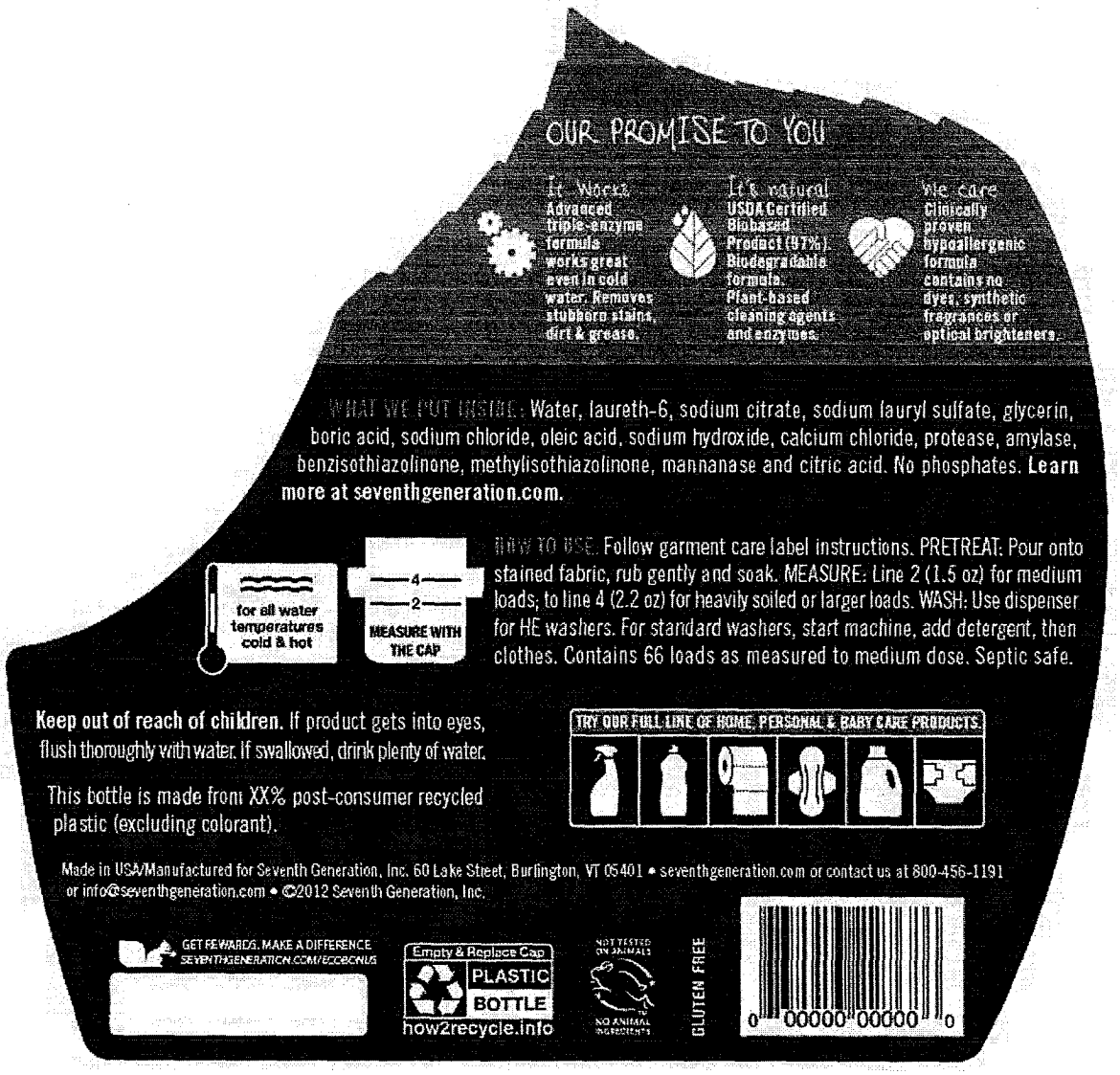
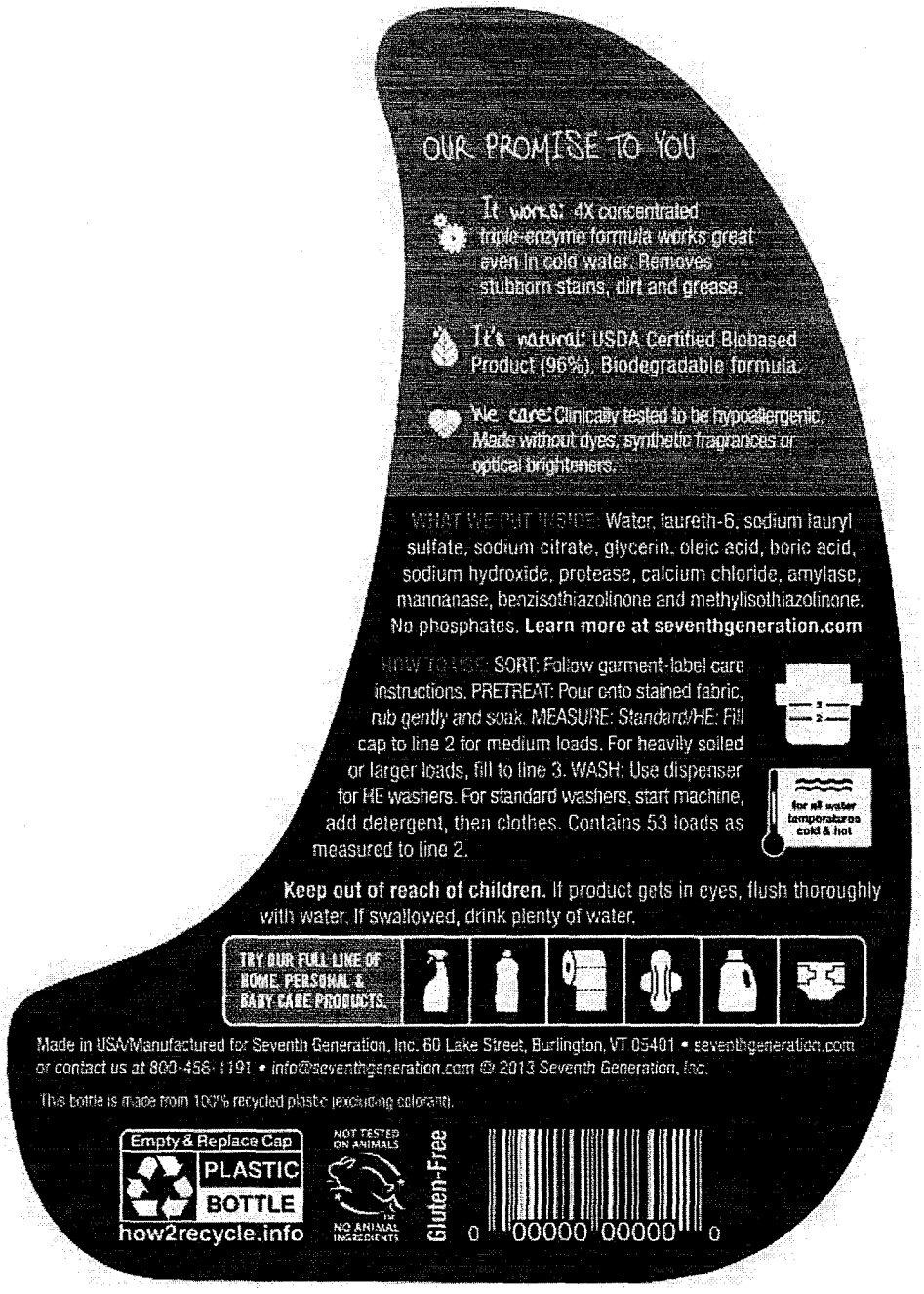

NEW
seventh generation
ULTRA POWER PLUS
WITH PlantTech GREASE CUTTING FORMULA
with PlantTech
20% MORE
CLEANING POWER*
NATURAL DISH LIQUID
22 FL OZ (0.37 PT) 650 mL

EXHIBIT D


OUR PROMISE TO YOU
It Works
Advanced triple-enzyme formula works great even in cold water. Removes stubborn stains, dirt & grease.
It's natural
USDA Certified Biobased Product (97%). Biodegradable formula. Plant-based cleaning agents and enzymes.
We care
Clinically proven hypoallergenic formula contains no dyes, synthetic fragrances or optical brighteners.
WHAT WE PUT INSIDE: Water, laureth-6, sodium citrate, sodium lauryl sulfate, glycerin, boric acid, sodium chloride, oleic acid, sodium hydroxide, calcium chloride, protease, amylase, benzisothiazolinone, methylisothiazolinone, mannanase and citric acid. No phosphates. Learn more at seventhgeneration.com.
for all water temperatures cold & hot
4
2
MEASURE WITH THE CAP
HOW TO USE: Follow garment care label instructions. PRETREAT: Pour onto stained fabric, rub gently and soak. MEASURE: Line 2 (1.5 oz) for medium loads; to line 4 (2.2 oz) for heavily soiled or larger loads. WASH: Use dispenser for HE washers. For standard washers, start machine, add detergent, then clothes. Contains 66 loads as measured to medium dose. Septic safe.
Keep out of reach of children. If product gets into eyes, flush thoroughly with water. If swallowed, drink plenty of water.
This bottle is made from XX% post-consumer recycled plastic (excluding colorant).
TRY OUR FULL LINE OF HOME, PERSONAL & BABY CARE PRODUCTS.
Made in USA/Manufactured for Seventh Generation, Inc. 60 Lake Street, Burlington, VT 05401 • seventhgeneration.com or contact us at 800-456-1191 or info@seventhgeneration.com • ©2012 Seventh Generation, Inc.
GET REWARDS. MAKE A DIFFERENCE.
SEVENTHGENERATION.COM/ECOBONUS
Empty & Replace Cap
PLASTIC BOTTLE
how2recycle.info
NOT TESTED ON ANIMALS
NO ANIMAL INGREDIENTS
GLUTEN FREE
0 00000 00000 0


OUR PROMISE TO YOU
It works: 4X concentrated triple-enzyme formula works great even in cold water. Removes stubborn stains, dirt and grease.
It's natural: USDA Certified Biobased Product (96%). Biodegradable formula.
We care: Clinically tested to be hypoallergenic. Made without dyes, synthetic fragrances or optical brighteners.
WHAT WE PUT INSIDE: Water, laureth-6, sodium lauryl sulfate, sodium citrate, glycerin, oleic acid, boric acid, sodium hydroxide, protease, calcium chloride, amylase, mannanase, benzisothiazolinone and methylisothiazolinone. No phosphates. Learn more at seventhgeneration.com
HOW TO USE: SORT: Follow garment-label care instructions. PRETREAT: Pour onto stained fabric, rub gently and soak. MEASURE: Standard/HE: Fill cap to line 2 for medium loads. For heavily soiled or larger loads, fill to line 3. WASH: Use dispenser for HE washers. For standard washers, start machine, add detergent, then clothes. Contains 53 loads as measured to line 2.
3
2
for all water temperatures cold & hot
Keep out of reach of children. If product gets in eyes, flush thoroughly with water. If swallowed, drink plenty of water.
TRY OUR FULL LINE OF HOME, PERSONAL & BABY CARE PRODUCTS
Made in USA/Manufactured for Seventh Generation, Inc. 60 Lake Street, Burlington, VT 05401 • seventhgeneration.com or contact us at 800-456-1191 • info@seventhgeneration.com © 2013 Seventh Generation, Inc.
This bottle is made from 100% recycled plastic (excluding colorant).
Empty & Replace Cap
PLASTIC BOTTLE
how2recycle.info
NOT TESTED ON ANIMALS
NO ANIMAL INGREDIENTS
Gluten-Free
0 00000 00000 0


OUR PROMISE TO YOU
IT WORKS.
Formulated with PlantTech™ for 25% more stain fighting power* to tackle our broadest range of stains. Deep cleans dirt and leaves clothes fresh and clean.
IT'S natural.
USDA Certified Biobased Product (91%).
WE CARE.
Clinically proven hypoallergenic. Non-toxic, biodegradable formula contains no dyes, synthetic fragrances or optical brighteners.
Plants are powerful, so say goodbye to those "impossible" stains thanks to the science of PlantTech™, our proprietary blend of advanced plant based enzymes. This ultra powerful stain-fighting formula deep cleans to remove our broadest range of stains and leaves your clothes fresh and clean. All that cleaning power, and it's hypoallergenic and non-toxic too!
WHAT WE PUT INSIDE: Water, laureth-6, sodium citrate, sodium lauryl sulfate, glycerin, boric acid, oleic acid, sodium chloride, sodium hydroxide, calcium chloride, protease, citral, citrus aurantium bergamia, citrus aurantium dulcis, citrus grandis, lavandula angustifolia, amylase, pectinase, benzisothiazolinone, methylisothiazolinone, mannanase and citric acid. d-Limonene is a component of this formula. No phosphates.
Learn more at seventhgeneration.com.
for all water temperatures cold & hot
HOW TO USE: Follow garment care label instructions. PRETREAT: Pour onto stained fabric, rub gently and soak. MEASURE: To the bottom of line 3 (1.75 oz) for medium loads; to the top of line 5 (2.6 oz) for heavily soiled or larger loads. WASH: Use dispenser for HE washers. For standard washers, start machine, add detergent, then clothes. Contains 54 loads as measured to medium dose. Septic safe.
Keep out of reach of children. If product gets into eyes, flush thoroughly with water. If swallowed, drink plenty of water.
NOT TESTED ON ANIMALS
NO ANIMAL INGREDIENTS
Empty & Replace Cap
PLASTIC BOTTLE
how2recycle.info
GLUTEN FREE
This bottle is made from 80% post-consumer recycled plastic (excluding colorant).
Made in USA/Manufactured for Seventh Generation, Inc. 60 Lake Street, Burlington, VT 05401
seventhgeneration.com or contact us at 800-211-4279 or info@seventhgeneration.com
©2014 Seventh Generation, Inc.
*compared to equal amount of our Original Natural Laundry Detergent
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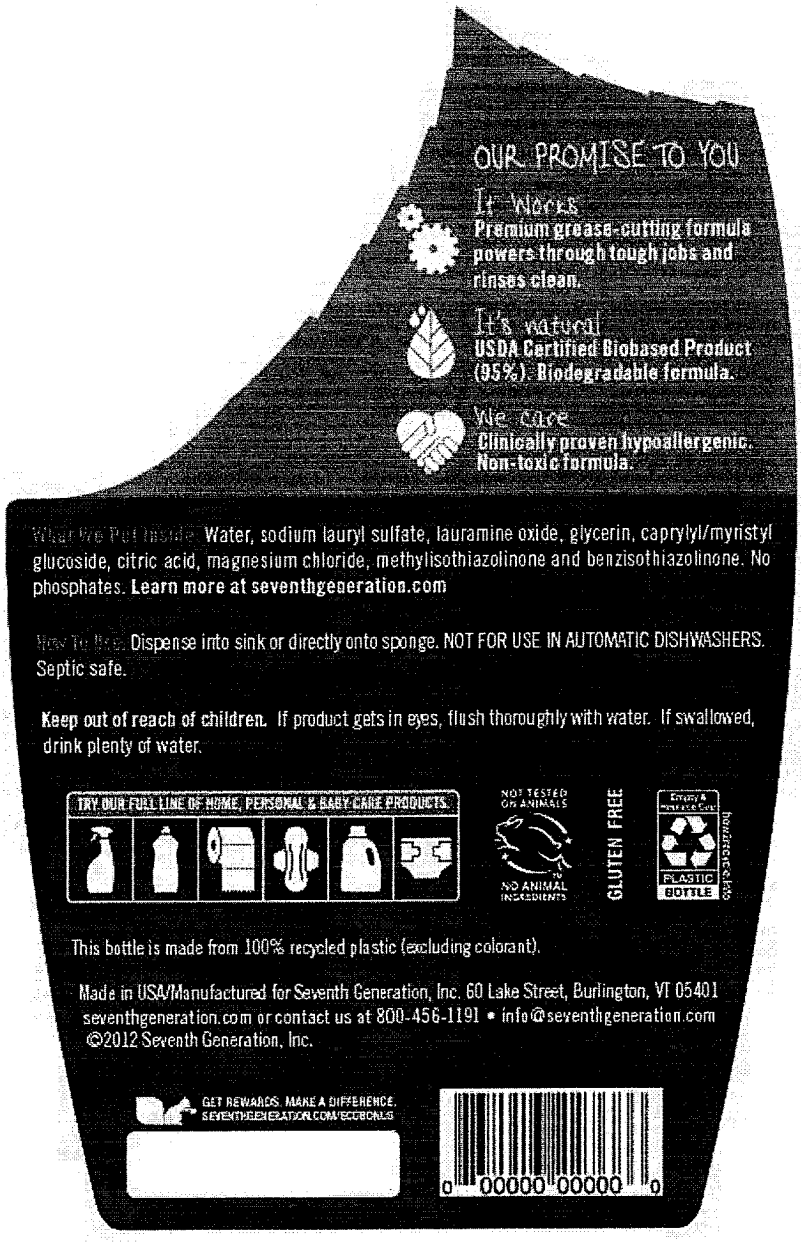
OUR PROMISE TO YOU
It Works
Premium grease-cutting formula powers through tough jobs and rinses clean.
It's natural
USDA Certified Biobased Product (95%). Biodegradable formula.
We care
Clinically proven hypoallergenic. Non-toxic formula.
What's Put Inside: Water, sodium lauryl sulfate, lauramine oxide, glycerin, caprylyl/myristyl glucoside, citric acid, magnesium chloride, methylisothiazolinone and benzisothiazolinone. No phosphates. Learn more at seventhgeneration.com
How To Use: Dispense into sink or directly onto sponge. NOT FOR USE IN AUTOMATIC DISHWASHERS. Septic safe.
Keep out of reach of children. If product gets in eyes, flush thoroughly with water. If swallowed, drink plenty of water.
TRY OUR FULL LINE OF HOME, PERSONAL & BABY CARE PRODUCTS
NOT TESTED ON ANIMALS
NO ANIMAL INGREDIENTS
GLUTEN FREE
PLASTIC BOTTLE
This bottle is made from 100% recycled plastic (excluding colorant).
Made in USA/Manufactured for Seventh Generation, Inc. 60 Lake Street, Burlington, VT 05401
seventhgeneration.com or contact us at 800-456-1191 • info@seventhgeneration.com
©2012 Seventh Generation, Inc.
GET REWARDS. MAKE A DIFFERENCE.
SEVENTHGENERATION.COM/ECOBONUS
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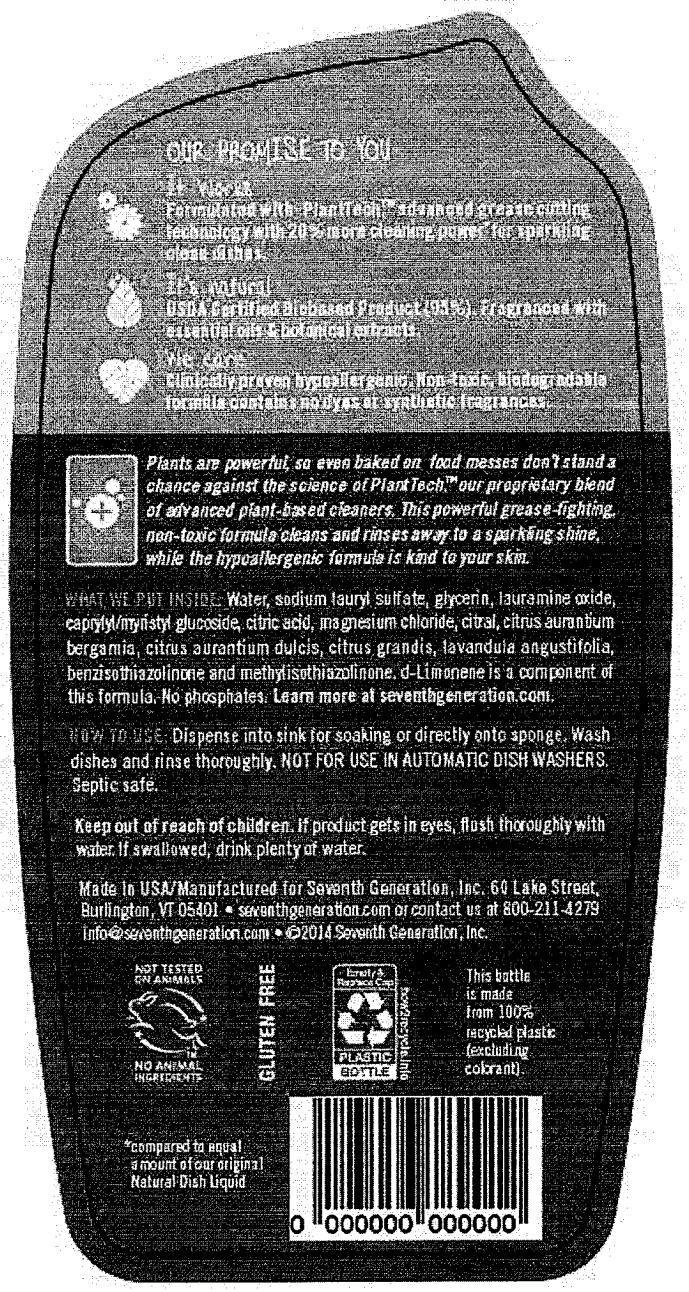
OUR PROMISE TO YOU
It Works
Formulated with PlantTech™ advanced grease cutting technology with 20%* more cleaning power for sparkling clean dishes.
It's Natural
USDA Certified Biobased Product (98%). Fragranced with essential oils & botanical extracts.
We Care
Clinically proven hypoallergenic. Non-toxic, biodegradable formula contains no dyes or synthetic fragrances.
Plants are powerful, so even baked on food messes don't stand a chance against the science of PlantTech™ our proprietary blend of advanced plant-based cleaners. This powerful grease-fighting, non-toxic formula cleans and rinses away to a sparkling shine, while the hypoallergenic formula is kind to your skin.
WHAT WE PUT INSIDE: Water, sodium lauryl sulfate, glycerin, lauramine oxide, caprylyl/myristyl glucoside, citric acid, magnesium chloride, citral, citrus aurantium bergamia, citrus aurantium dulcis, citrus grandis, lavandula angustifolia, benzisothiazolinone and methylisothiazolinone. d-Limonene is a component of this formula. No phosphates. Learn more at seventhgeneration.com.
HOW TO USE: Dispense into sink for soaking or directly onto sponge. Wash dishes and rinse thoroughly. NOT FOR USE IN AUTOMATIC DISH WASHERS. Septic safe.
Keep out of reach of children. If product gets in eyes, flush thoroughly with water. If swallowed, drink plenty of water.
Made in USA/Manufactured for Seventh Generation, Inc. 60 Lake Street, Burlington, VT 05401 • seventhgeneration.com or contact us at 800-211-4279 info@seventhgeneration.com • ©2014 Seventh Generation, Inc.
NOT TESTED ON ANIMALS
NO ANIMAL INGREDIENTS
GLUTEN FREE
Recycle if Replaced Cap
PLASTIC BOTTLE
Recyclable
This bottle is made from 100% recycled plastic (excluding colorant).
*compared to equal amount of our original Natural Dish Liquid
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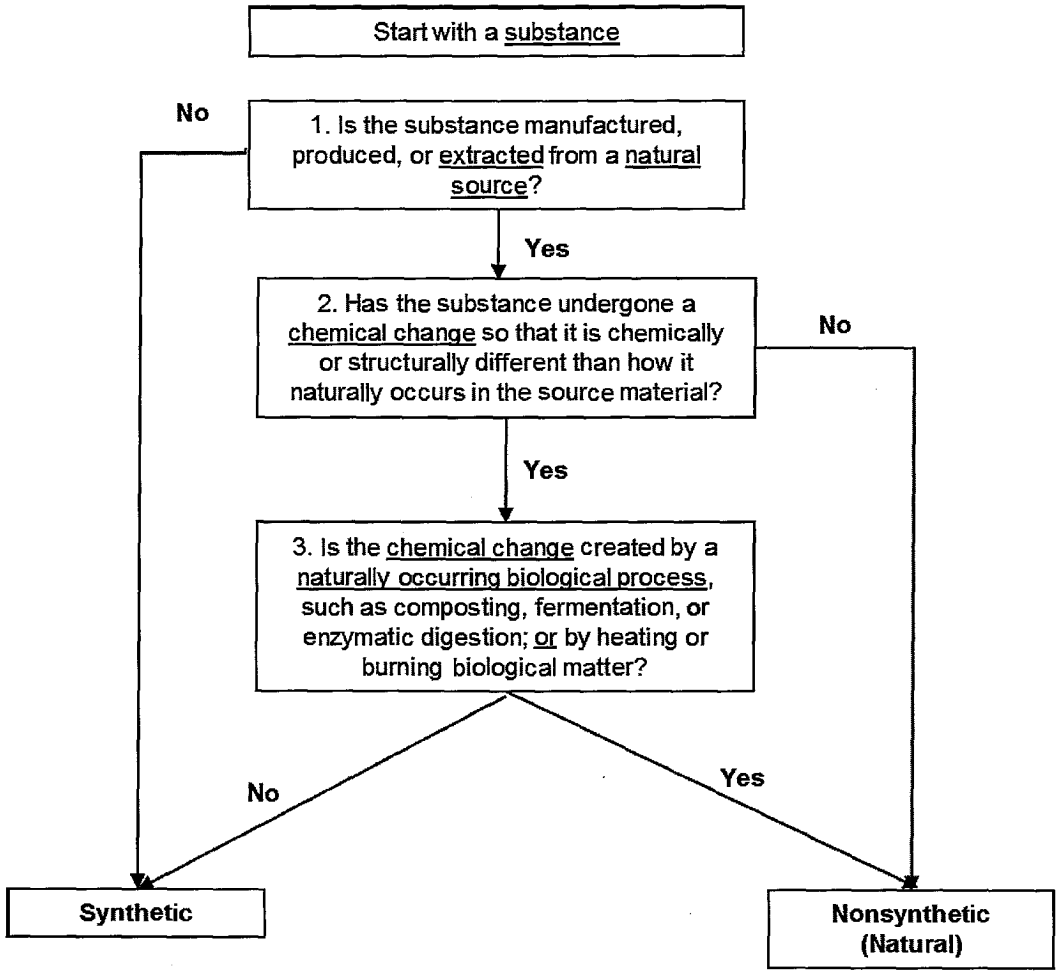
EXHIBIT E

Draft Guidance
Decision Tree for Classification of Materials as Synthetic or Nonsynthetic

Underlined terms defined on page 2



Definitions (bolded terms in 7 CFR 205.2)

Agricultural inputs. All substances or materials used in the production or handling of organic agricultural products.

Agricultural product. Any agricultural commodity or product, whether raw or processed, including any commodity or product derived from livestock, that is marketed in the United States for human or livestock consumption.

Allowed synthetic. A substance that is included on the National List of synthetic substances allowed for use in organic production or handling.

Chemical change. A process (i.e. chemical reaction) whereby a substance is transformed into one or more other distinct substances.

Extract. To separate, withdraw, or obtain one or more constituents of an organism, substance, or mixture by use of solvents (dissolution), acid-base extraction, or mechanical or physical methods.

Formulate. To combine different materials according to a recipe or formula.

Generic. The common and familiar non-proprietary name.

Manufacture. To make a substance from raw materials.

Natural source. Naturally occurring mineral or biological matter.

Naturally occurring biological process. A process that occurs due to the action of biological organisms or subcomponents of biological organisms, such as enzymes. Examples of naturally occurring biological processes include, but are not limited to, fermentation, composting, manure production, enzymatic processes, and anaerobic digestion.

Nonagricultural substance. A substance that is not a product of agriculture, such as a mineral or a bacterial culture, that is used as an ingredient in an agricultural product. For the purposes of this part, a nonagricultural ingredient also includes any substance, such as gums, citric acid, or pectin, that is extracted from, isolated from, or a fraction of an agricultural product so that the identity of the agricultural product is unrecognizable in the extract, isolate, or fraction.

Nonsynthetic (natural). A substance that is derived from mineral, plant, or animal matter and does not undergo a synthetic process as defined in section 6502(21) of the Act (7 U.S.C. 6502(21)). For the purposes of this part, nonsynthetic is used as a synonym for natural as the term is used in the Act.

Substance. A generic type of material, such as an element, molecular species, or chemical compound, that possesses a distinct identity (e.g. having a separate Chemical Abstracts Service

(CAS) number, Codex International Numbering System (INS) number, or FDA or other agency standard of identity).

Synthetic. A substance that is formulated or manufactured by a chemical process or by a process that chemically changes a substance extracted from naturally occurring plant, animal, or mineral sources, except that such term shall not apply to substances created by naturally occurring biological processes.

Table 1. Classification examples of inputs:

Substance	Classification	Explanation
Ash (burned wood)	Nonsynthetic	Substance is created by burning biological matter.
Calcium carbonate (limestone)	Nonsynthetic	Substance is produced from a natural source (mined mineral) and does not undergo chemical change.
Calcium oxide (quicklime)	Synthetic	Substance is produced from a natural source (mined mineral), but undergoes chemical change caused by heating the mineral.
Citric acid	Nonsynthetic	Substance is created from a naturally occurring biological process (microbial fermentation of carbohydrate substances).
Enzymes, without synthetic additional ingredients	Nonsynthetic	Substance is extracted from a natural source and is not formulated with synthetic ingredients
Gibberellic acid	Nonsynthetic	Substance is extracted from a natural source without further chemical change
Liquid fish products – pH adjusted with phosphoric acid	Synthetic	Substance is derived from a natural source, but is treated with synthetic acids for pH adjustment.
Molasses	Nonsynthetic	Substance is derived from a natural source and chemical change is due to heating or naturally occurring biological processes.
Newspaper	Synthetic	Substance is manufactured via a chemical process.
Raw manure	Nonsynthetic	Substance is from a natural source and used without further processing.
Rosemary oil	Nonsynthetic	Substance is extracted from a natural source.

EXHIBIT F

United States Environmental Protection Agency | Prevention, Pesticides And Toxic Substances (7508C) | EPA-738-F-98-008 October 1998

EPA

R.E.D. FACTS

Methylisothiazolinone

Pesticide Reregistration

All pesticides sold or distributed in the United States must be registered by EPA, based on scientific studies showing that they can be used without posing unreasonable risks to people or the environment. Because of advances in scientific knowledge, the law requires that pesticides which were first registered before November 1, 1984, be reregistered to ensure that they meet today's more stringent standards.

In evaluating pesticides for reregistration, EPA obtains and reviews a complete set of studies from pesticide producers, describing the human health and environmental effects of each pesticide. The Agency develops any mitigation measures or regulatory controls needed to effectively reduce each pesticide's risks. EPA then reregisters pesticides that can be used without posing unreasonable risks to human health or the environment.

When a pesticide is eligible for reregistration, EPA explains the basis for its decision in a Reregistration Eligibility Decision (RED) document. This fact sheet summarizes the information in the RED document for reregistration case 3092, methylisothiazolinone. The Reregistration Eligibility Decision covers the two active ingredients 5-chloro-2-methyl-3(2H)-isothiazolone and 2-methyl-3(2H)-isothiazolone. These two active ingredients occur together in the currently registered products in approximately a 3:1 ratio, respectively, and are commonly referred to as methylisothiazolinone.

Use Profile

Methylisothiazolinone is used to control slime-forming bacteria, fungi, and algae in pulp/paper mills, cooling water systems, oil field operations, industrial process waters, and air washer systems and is incorporated into adhesives, coatings, fuels, metal working fluids, resin emulsions, paints, and various other speciality industrial products as a preservative. It is also used to control the growth of mold, mildew, and sapstain on wood products. Formulations include soluble concentrated liquids and soluble concentrated solids. Products containing methylisothiazolinone are added to systems and industrial products using manual pouring and metered pumping methods, dip tanks and sprayers. Use practice limitations include National Pollutant Discharge Elimination System (NPDES) license restrictions.

Regulatory History

Methylisothiazolinone was first registered in the U.S. in 1977 as an antimicrobial with various uses. There are currently 85 products registered including one technical product.

In 1987 the Agency issued the Antimicrobial Data Call-In Notice to registrants with pesticides containing methylisothiazolinone to obtain additional chronic and subchronic toxicity data. A Phase 4 Data Call-In was issued on November 3, 1992, requiring additional toxicity and environmental fate data.

Human Health Assessment

Toxicity

In studies using laboratory animals, methylisothiazolinone has been shown to be of moderate acute toxicity by the oral and inhalation routes. It is highly acutely toxic when applied dermally or to the eye and is considered to be corrosive.

In subchronic studies, the most significant toxicological effect was microscopic lesions in the nasal turbinates from inhalation exposure. Developmental and chronic feeding/carcinogenicity studies in rats resulted in no significant effects and the Agency classified methylisothiazolinone as a Group D chemical, not classifiable as to human carcinogenicity. Results from mutagenicity studies were equivocal.

Dietary Exposure

Tolerances or residue limits are established for methylisothiazolinone in adhesives, paper, and paper products which may contact food. These uses are regulated by the U.S. Food and Drug Administration (FDA). There are no other registered food uses of methylisothiazolinone.

Occupational and Residential Exposure

Based on current use patterns, handlers may be exposed to methylisothiazolinone during and after normal use of the liquid and solid soluble concentrate formulations. Persons in residential settings may be exposed to products containing methylisothiazolinone. Therefore, an exposure assessment was conducted based on the toxicological endpoint of the respiratory effect from the subchronic inhalation study.

The open-pouring application of methylisothiazolinone is considered the worst-case inhalation exposure scenario for applicators. The worst-case scenario for persons exposed to methylisothiazolinone-treated products is the paint application use.

Although exposures to workers in areas where products containing methylisothiazolinone have recently been applied are expected, EPA believes that these post-application exposures would be significantly less than those for handlers applying the pesticide.

There are no methylisothiazolinone products labeled for homeowner use. Exposures to homeowners may occur from products, such as adhesives, paints or paper products, treated with methylisothiazolinone. Again, the Agency believes that these exposures would be minimal.

Human Risk Assessment

Methylisothiazolinone is moderately to highly acutely toxic in oral, dermal, eye irritation, dermal irritation, and inhalation acute toxicity studies.

The use of methylisothiazolinone in the manufacture of paper, paperboard, and adhesives which may contact food is regulated by FDA. There are no other registered food uses.

The Agency concluded that the risks of short-term and intermediate-term occupational exposure to pesticide handlers are acceptable. Margins of Exposure (MOEs) for all uses were above 100. An MOE of less than 100 is of concern to the Agency. Short-term risks of corrosivity can be adequately managed through the use of personal protective equipment (PPE) and monitoring, as necessary. The Agency further believes risks from secondary occupational exposures, residential exposures, and post-application exposures are comparatively less and also acceptable. However, protective measures are being imposed including additional product specific PPE (when appropriate), and baseline PPE.

Environmental Assessment

Environmental Fate

Of the two chemicals (5 -chloro-2-methyl-3(2H)-isothiazolone and 2-methyl-3(2H)-isothiazolone) that compose methylisothiazolinone, only 5 -chloro-2-methyl-3(2H)-isothiazolone was susceptible to hydrolysis and only at alkaline pH. 5-Chloro-2-methyl-3(2H)-isothiazolone was very mobile in most soils. The degradation profile observed in an aqueous availability study is similar to that observed in the hydrolysis studies.

Ecological Effects

Methylisothiazolinone is moderately to practically non-toxic to birds, and moderately to highly toxic to freshwater and estuarine/marine organisms.

Ecological Effects Risk Assessment

While the hazard to aquatic organisms from methylisothiazolinone has been characterized, a quantitative risk assessment has not been conducted. The risks to aquatic environments from this use are regulated under the NPDES permitting program of EPA's Office of Water. The Agency currently requires that labels for all methylisothiazolinone products require that discharges to aquatic environments comply with an NPDES permit.

Risk Mitigation

To lessen the potential human health risks posed by methylisothiazolinone, EPA is requiring the following risk mitigation measures.

(1) The Agency is establishing active-ingredient based minimum PPE for primary occupational handlers. Since all the MOEs generated are based on units of exposure from the Pesticide Handlers Exposure Database in which handlers wore chemical resistant gloves, long-sleeve shirts, long pants, and shoes plus socks, these PPE are required for occupational handlers of methylisothiazolinone products.

(2) The acute dermal, inhalation and ocular toxicity of the end-use products will be used to determine appropriate protection from the corrosivity of methylisothiazolinone.

Additional Data Required

EPA has required additional generic information describing the hydrolysis of 5-chloro-2-methyl-3(2H)-isothiazolone at pH 9 to confirm its regulatory assessments and conclusions.

The Agency also is requiring methylisothiazolinone product-specific data including product chemistry and acute toxicity studies, revised Confidential Statements of Formula (CSFs), and revised labeling for registration.

Product Labeling Changes Required

All methylisothiazolinone end-use products must comply with EPA's current pesticide product labeling requirements and with the following. For a comprehensive list of labeling requirements, please see the methylisothiazolinone RED document.

Personal Protective (PPE) Requirements

(1) EPA is establishing the following minimum, baseline PPE: Mixers, loaders, and others exposed to methylisothiazolinone products must wear:

-- Long-sleeve shirt and long pants,

-- Chemical resistant gloves,

-- Shoes plus socks.

(2) If the end-use product is classified as Toxicity Category I or II for eye irritation potential, add to the above PPE:

--Protective eyewear

(3) If the end-use product is classified as Toxicity Category I or II for acute dermal toxicity or skin irritation potential, add:

--Chemical-resistant apron

(4) If the end-use product is classified as Toxicity Category I or II for acute inhalation toxicity, add:

--Respirator (the type must be specified; EPA will assist registrants in determining appropriate respirators during product reregistration).

Labeling Clarifications

The following clarifications must be made on all end-use products labels, where applicable.

(1) Use Profile Clarifications

Registrants must specify on labeling of products containing methylisothiazolinone the complete directions for use for each use pattern: site of application, type of application, timing of application, equipment used for application, and the rate of application (dosage).

(2) Use on Pilings

Methylisothiazolinone is to be used only on terrestrial-use pilings not aquatic-use pilings. The phrase "terrestrial-use pilings" must be used when referring to any type of piling.

(3) Water Treatment Systems

All uses of products containing methylisothiazolinone in water treatment systems must clearly specify recirculating water treatment systems. The term "recirculating" must be added before all references to water treatment systems (e.g., water treatment, cooling towers, etc.).

(4) Clarification of Oil Drilling Mud Use

To clarify the intent of the oil recovery drilling muds/packer fluids use (as an aquatic or terrestrial non-food use pattern), the following statement must be added to the labels for terrestrial non-food oil drilling muds and packer fluids:

"For use in terrestrial wells only."

And the following statement must be added to the precautionary labeling:

"Do not apply in marine and/or estuarine oil fields."

The following statement must be added to the labels for aquatic non-food industrial oil drilling muds and packer fluids:

"For use in offshore wells only."

For use in both terrestrial and offshore oil drilling muds and packer fluids, the following statement must be added:

"This product may be used for terrestrial and off-shore oil drilling muds and packer fluids."

Other Labeling Requirements

The Agency is requiring the following labeling statements to be located on all end-use products containing methylisothiazolinone that are intended primarily for occupational use.

(1) Application Restrictions

"Do not apply this product in a way that will contact workers or other persons."

(2) User Safety Requirements

"Discard clothing or other absorbent materials that have been drenched or heavily contaminated with this product's concentrate. Do not reuse them. Follow manufacturer's instructions for cleaning/maintaining PPE. If there are no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry."

(3) User Safety Recommendations

- "Users should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet."
- "Users should remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing."
- "Users should remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible wash thoroughly."

(4) Skin Sensitizer Statement

"This product may cause skin sensitization reactions in some people."

Regulatory Conclusion

The use of currently registered products containing methylisothiazolinone in accordance with approved labeling and as described in the Reregistration Eligibility Decision Document will not pose unreasonable risks or adverse effects to humans or the environment. Therefore, all uses of these products are eligible for reregistration.

Methylisothiazolinone products will be reregistered once the required product-specific data, generic data, revised Confidential Statements of Formula, and revised labeling are received and accepted by EPA.

For More Information

EPA is requesting public comments on the Reregistration Eligibility Decision (RED) document for methylisothiazolinone during a 60-day time period, as announced in a Notice of Availability published in the Federal Register. To obtain a copy of the RED document or to submit written comments, please contact the Pesticide Docket, Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs (OPP), US EPA, Washington, DC 20460, telephone 703-305-5805.

Electronic copies of the RED and this fact sheet can be downloaded from the Pesticide Special Review and Reregistration Information System at 703-308-7224. They also are available on the Internet using ftp on *FTP.EPA.GOV*, or using WWW (World Wide Web) on *WWW.EPA.GOV*.

Printed copies of the RED and fact sheet can be obtained from EPA's National Center for Environmental Publications and Information (EPA/NCEPI), PO Box 42419, Cincinnati, OH 45242-0419, telephone 513-489-8190, fax 513-489-8695.

Following the comment period, the methylisothiazolinone RED document also will be available from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, telephone 703-487-4650.

For more information about EPA's pesticide reregistration program, the methylisothiazolinone RED, or reregistration of individual products containing methylisothiazolinone, please contact the Special Review and Reregistration Division (7508C), OPP, US EPA, Washington, DC 20460, telephone 703-308-8000.

For information about the health effects of pesticides, or for assistance in recognizing and managing pesticide poisoning symptoms, please contact the National Pesticides Telecommunications Network (NPTN). Call toll-free 1-800-858-7378, between 9:30 am and 7:30 pm Eastern Standard Time, Monday through Friday.

EXHIBIT G

CONTACT ALLERGEN OF THE YEAR

Methylisothiazolinone

Mari Paz Castanedo-Tardana, MD* and Kathryn A. Zug, MD*†

The preservative methylisothiazolinone (MI) is the American Contact Dermatitis Society Contact Allergen of the Year for 2013. Because the use of MI in cosmetics and toiletries in the United States rises, MI exposure also rises. Although it might seem likely that testing with methylchloroisothiazolinone (MCI)/MI would be adequate to pick up contact allergy to MI alone, the mix misses approximately 40% of allergy to MI, likely because of the low concentration of MI in the MCI/MI combination patch test. In Europe, several groups have documented frequency of allergy to this preservative of approximately 1.5%. The frequency of allergy to this preservative in the United States is unknown. If you are not testing for allergy to this preservative, you may be overlooking the importance of a very relevant preservative allergen that, to date, has managed to stay under the radar in the United States. This report reviews the background and reasons for adding MI to our routine screening patch testing series.

If you are not testing it, it is likely that you are missing allergy to it. Methylisothiazolinone (MI) is a preservative whose use is on the rise. More opportunities for exposure mean more opportunities for contact allergy to this preservative, which is categorized as a moderate-strong sensitizer.[1,2] Methylisothiazolinone was approved for use as a preservative in cosmetics in 2005 at a maximum concentration of 100 ppm (0.01%). Since then, a striking rising use of this preservative in cosmetic, toiletry, and sunscreen products can be appreciated. In the European Union, where recent investigations of MI allergy have been well described, frequency of allergy to this preservative is already reported to be at the same level as other preservatives that have been on the market for numerous years.[3] Because methylisothiazolinone is not routinely tested in any standard screening series, it is likely that allergy to this preservative is being widely overlooked.

A Short History of Methylchloroisothiazolinone/Methylisothiazolinone and Methylisothiazolinone

Methylchloroisothiazolinone (MCI) and MI in a 3:1 combination (MCI/MI; trade names: Kathon CG, Euxyl K 400) is a widely used preservative in both industrial and consumer products. Animal and clinical studies have shown that both MCI and MI can cause contact allergy; MCI is the more potent allergen in this combination.[4] MCI/MI is one of the most common causes of preservative contact allergy and dermatitis.[5] North America Contact Dermatitis Group (NACDG) data from 2009 to 2010 show that MCI/MI (100 ppm aq) had a 2.5% frequency of positive reactions of 4302 patients tested. Thisallergen was the fifth most commonly positive preservative behind formaldehyde 1% aq (5.8% positive), quaternium 15.2% pet (5.8% positive), iodopropynyl butyl carbamate 0.5% pet (4.3% positive), and methyldibromoglutaronitrile/phenoxyethanol 2.0% pet (3.8% positive). Of these 5 preservatives, the relevance assigned to MCI/MI was the highest; definite and probable relevance was 54.6%, whereas possible relevance was 38.9%.[6]

Rates of contact allergy to MCI/MI rose to levels of up to 8% when it was first introduced as a preservative in 1980.[7,8] This was thought to be secondary to high concentrations found in "leave-on" cosmetic products; this prompted more strict use concentration recommendations from expert panels in both the European Union and the United States. In 1989, the Cosmetics Directive of the European Union considered the recommendations given by the Scientific Committee on Consumer Safety and limited the concentration of MCI/MI to 15 ppm in both "leave-on" and "rinse-off" products.[9] A few years later, in 1992, the US Cosmetic Ingredient Review recommended an even lower concentration limit of 7.5 ppm MCI/MI in "leave-on" cosmetic products.[10] Despite these modifications in use concentrations, sensitization prevalence to MCI/MI has remained fairly stable at 1% to 4%, according to the European Surveillance System on Contact Allergy Network.[11] In the United States, the NACDG data indicate a stable frequency of positive reactions to MCI/MI from between 2.2% and 3.6% over the period from 2001 to 2010.[6] The most recent NACDG data show a frequency of 2.5%; this is similar to lanolin alcohol, diazolidinyl urea, and imidazolidinyl urea frequency of positive patch reactions in this selected population of patch-tested patients.[6]

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Methylisothiazolinone (technical name/synonym: 2-methyl-4-isothiazolin-3-one; CAS no.: 2682-20-4; Trade names: Neolone 950, Microcare MT, OriStar MIT, RH-24,573, and the industrial microbicides: Kordek 573T and RH-573T)[12] constitutes 25% of MCI/MI, thus a maximum MI concentration of 3.5 ppm in all rinse-off products and 1.8 ppm in leave-on products sold in the United States.

Because MI is believed to be a weaker sensitizer than MCI, in the early 2000s, MI *alone* was released as an individual preservative for industrial products. In 2005, MI was approved for use as a preservative in cosmetics and household products. Both the Cosmetic Ingredient Review in the United States and the European Scientific Committee on Consumer Safety evaluated MI—which alone is a less effective biocide than MCI/MI—and concluded that 100 ppm was a safe concentration to use in cosmetics.[13] The introduction of MI alone has therefore resulted in a more than 25-times increase in the permitted concentration of MI in cosmetics from 3.75 ppm in rinse-off and 1.8 ppm in leave-on products to 100 ppm.[13] Currently, no limitations exist for the concentration of MI in industrial products.

Methylisothiazolinone Sensitization

Based on animal local lymph node assays, Basketter et al[14] categorized MI as a moderate sensitizer. However, the United Nations Globally Harmonized System of Classification and Labeling of Chemicals have classified MI as a strong allergen.[15] Rohm and Haas (now Dow Chemicals, Midland, Mich) has performed a series of both animal and human studies to assess the sensitization potential of MI. They concluded that MI sensitized only at high concentrations (>1000 ppm).[16] In 1994, Rohm and Haas evaluated the sensitization potential of 98% MI using repeated insult patch test studies. Eighty human subjects were tested with 5 test concentrations of MI (50, 100, 250, 500, and 1000 ppm). Methylisothiazolinone was applied for 23 hours daily for 21 consecutive days. After a 10- to 14-day rest period, the subjects were challenged for 23 hours. At challenge, 2 subjects in the 1000-ppm dose group had mild reactions and were considered sensitized. The authors concluded that the sensitization threshold for 98% MI was at or approximately 1000 ppm.[16] In a later series of repeated insult patch test studies also performed by Rohm and Haas, the sensitization potential of 50% MI was evaluated at 200, 300, 400, 500, and 600 ppm. During the induction phase, MI was applied 3 times a week for 3 weeks with occlusive patches for 24 hours. After a 7- to 15-day rest period, subjects were challenged on a virgin site for 24 hours with the same induction concentration. Only 1 subject in each of the 400-ppm and 500-ppm dose groups had an erythema response. The authors concluded that up to a concentration of 600 ppm, MI is not a dermal sensitizer.[16]

Methylisothiazolinone Elicitation

In a small repeat open application test (ROAT) study performed by Lundov et al,[17] 11 MI-allergic patients were exposed to MI via a ROAT test that resembled the use of a leave-on cream preserved with 5, 50, and 100 ppm of MI. Of MI allergic patients, 64% reacted to 100 and 50 ppm, whereas 18% also reacted to the 5-ppm–containing cream used in this ROAT test. Importantly, the authors concluded that the eliciting concentration for MI can be as low as 5 ppm.

Contact Allergy to MI Alone

The first occupational cases of MI contact allergy were reported by Isaksson et al[18] in 2004 and Thyssen et al[19] in 2006, from occupational exposure to a wallpaper glue and paint, respectively. In 2010, Garcia-Gavin et al[20] published the first 7 nonoccupational cases stemming from the use of cosmetics/toiletries that contained MI alone. Of the 7 patients, 6 had perianal dermatitis from the use of MI-containing moist toilet paper, whereas the remaining patient had eyelid dermatitis from the use of an MI-containing makeup remover.

Subsequently, more toiletry-related cases of allergy to MI have been reported.[21] In a retrospective study by Lundov et al[3] in which 2536 Danish patients were patch tested with MI alone, a history of exposure to the allergen via cosmetics (both rinse-off and leave-on) was identified in 32% of the MI-positive cases. Hair care products were the most frequent cause of cosmetic exposure to MI in this study. Furthermore, cases of airborne contact dermatitis after exposure to MI (from carpet glue and paint for example) continue to emerge in the literature.[20,22,23]

Sources of Exposure

Exposure to MI can derive from both cosmetic and occupational sources. Methylisothiazolinone can be found as a preservative in many cosmetic products, including baby products (lotion, oils, powders, and creams), bath products (soaps, detergents, and bubble bats), makeup (eyeliners, eye makeup remover, blushes, and face powders), hair care products (shampoo, conditioners, sprays, straighteners, rinses, and wave sets), hair-coloring products (dyes and colors, tints, and bleaches), nail care products, deodorants, shaving products (after shaves and shaving creams), skin care products (cleansers, creams, lotions, and moisturizers), suntan products, and sunscreens among others. Importantly, in the United States, full ingredient labeling is not always the case for sunscreen products, making recognition of MI among this type of product difficult for both the consumer, the patient, and their physician. Our first case of MI allergy occurred in a woman whom we suspected to have a sunscreen allergy; testing result to a sunscreen tray and the NACDG 2011-2012 allergen tray (including MCI/MI at 100 ppm) was entirely negative; testing to the sunscreen product itself was positive. The sunscreen contained MI, and further testing to 2000 ppm MI confirmed this allergen as causative in this patient's severe, recurrent dermatitis of the face, neck, and arms.

According to information supplied to the US Food and Drug Administration by industry as part of the Voluntary Cosmetic

Ingredient Registration Program, MI was used in a total of 1125 cosmetic products in the United States in 2007.[24] (The information provided under the Voluntary Cosmetic Ingredient Registration Program, however, does not clearly indicate whether MI was used alone or in concomitance to MCI.) Of the 1125 products, 24% (n = 275) were shampoos, 18% (n = 206) were conditioners, and 10% (n = 117) were baby soaps and detergents. This illustrates that most products preserved with MI are in the rinse-off category. Wet wipes (baby wipes, moist towelettes, and moist toilet paper) for intimate hygiene are a well-identified sensitization source for MI; use of such leave-on products in nonkeratinized and occluded skin likely enhances penetration of this allergen.[21] These wipes are not only used for babies, but they are also used in adults, and frequently in the aged or infirm, in circumstances when bathing is more difficult.

Further Food and Drug Administration data on the frequency of preservative use in cosmetic products show that the use of MI is on the rise. Recent data show that, in 2010, a total of 2408 US cosmetic products were preserved with MI alone.[25] This means that the number of cosmetic products preserved with MI more than doubled from 2007 to 2010 (from 1125 to 2408 cosmetic products).

Actual use concentration of MI in products is a critical sensitization risk factor. An industry survey conducted in 2008 showed that the concentration of MI used to preserve cosmetics ranged from 0.000004% to 0.01% (0.01% = 100 ppm).[26]

Occupational sources of MI include paints, inks, glues, lacquers, varnishes, and cutting oils, among other industrial products. A recent study by Lundov et al[3] showed that painters constitute the majority (nearly half) of occupational allergic contact dermatitis cases to MI alone. It is reasonable then to assume that MI is frequently used in paints. Currently, there is no maximum permitted concentration and no labeling requirements for MI in industrial products, including paints, which makes it difficult for allergic individuals to avoid contact with this allergen.[22]

Household products containing MI include dishwashing liquid soaps,[27] laundry detergents, laundry stain removers, fabric softeners, all purpose cleansers, glass cleaners, and wood cleansers among others. Surprisingly, we have found that MI is also contained in some popular "green" household cleaning products.

Prevalence of MI Contact Allergy

Because MI is not routinely tested in the United States, there is no US data reporting the frequency of positive reactions to this allergen. Three European groups have investigated the prevalence of MI contact allergy in patch test patients; despite using different patch test concentrations, the overall prevalence of contact allergy to MI in Europe has been shown to be in the area of 1.4% to 1.54% (Table 1).

The Danish group[3] patch tested 2536 patients with dermatitis with the European baseline series supplemented with MI (0.2% = 2000 ppm aq) from May 2006 to February 2010. The overall prevalence of MI contact allergy in this study was 1.5%, making MI the fourth most common preservative contact allergen in Denmark. According to the MOAHLFA index (male, occupational dermatitis, atopic dermatitis, hand eczema, leg dermatitis, facial dermatitis, age older than 40 years) calculated in this study, MI contact allergy was more often associated with occupational exposure, hand eczema, and age older than 40 years. Besides hand eczema, the anatomical sites of dermatitis included the face, the arms, and the legs.

The German group[28] patch tested 13,433 patients to MI (0.05% aq = 500 ppm) from 2005 to 2009. Of 13,433 patients, 215 had a positive patch test reaction to MI, a prevalence of 1.54%. Similar to the Danish study, MI was associated with occupational dermatitis in which hands were often affected.

The Finnish group15 patch tested 10,821 patients to 2 different concentrations of MI—0.1% (1000 ppm) and 0.03% (300 ppm) from 2006 to 2008. 1.4%—and 0.6% showed a positive patch test reaction to 0.1% and 0.03%, respectively.

The Problem

Few data exist concerning the cross-reaction patterns of MCI and MI. Bruze et al[4] showed that a *small* proportion of patch test subjects sensitized to MCI/MI also reacted to MI. Isaksson et al[29] also suggested that patients with high patch test reactivity to MCI may also react to high concentrations of MI (1000 ppm).

The percentage of concomitant reactions between MCI/MI and MI in the patch test population is relevant with regard to the diagnosis of MI-contact allergy. In the Danish study,[3] only 40% of patients with a positive reaction to MI reacted also to MCI/MI. In the Finnish study,[15] 66% of MI positives reacted to MCI/MI, and in the German study,[25] 67% of patients with a positive reaction to MI also had a positive reaction to MCI/MI. This means that by patch testing to MCI/MI but not MI alone, MI allergy could be missed in 33% to 60% of the cases. This is likely because of the low

TABLE 1. Prevalence of MI Contact Allergy

Author	Lundov et al[3]	Schnuch et al[28]	Ackermann et al[15]
Country	Denmark	Germany	Finland
Years	2006–2010	2005–2009	2006–2008
Prevalence, no. MI-positive cases/n = prevalence	37/2536 = 1.5%	215/13,433 = 1.54%	147/10,821 = 1.4%
MI patch test concentration (aq)	2000 ppm (0.2%)	500 ppm (0.05%)	1000 ppm (0.1%), 300 ppm (0.03%)

concentration of MI in the MCI/MI patch test substance (75 ppm MCI/25 ppm MI).

Patch Testing to MI

Experience with MI patch test concentrations is still limited. In the most recent report of the patch test results from the European Surveillance System on Contact Allergy Network[11] published in July 2012, their members concluded that "MI warrants consideration for inclusion into the European Baseline Series." They, however, did not comment on the ideal patch test concentration for MI.

As noted previously, MI has been tested at 300 ppm (0.03% aq), 500 ppm (0.05% aq), 1000 ppm (0.1% aq), and 2000 ppm (0.2% aq), all yielding a relatively similar percentage of positive patch test reactions. More studies are needed to establish the optimal patch test concentration for this allergen. As with any other allergen, the ideal patch test concentration should be able to detect as many cases of contact allergy as possible without causing irritant reactions or active sensitization.[30] Preliminary results from Denmark suggest that 2000 ppm (0.2% aq) may be an appropriate patch test concentration.[13] Methylisothiazolinone is commercially available at 2000 ppm from Chemotechnique Diagnostics, Vellinge, Sweden, in a standard preparation.

CONCLUSIONS

Methylisothiazolinone alone is an important emerging "new" preservative allergen. It should be on the same suspect allergen list that would include quaternium 15, methylchloroisothiazolinone/methylisothiazolinone, diazolidinyl urea, imidazolidinyl urea, and iodopropynyl butylcarbamate, to name a few other important preservative allergens. It is concerning that a positive reaction to this allergen could be missed by testing with MCI/MI and not MI alone. Methylisothiazolinone should be considered as a potential suspect allergen among patients with suspected cosmetic dermatitis, facial dermatitis, and sunscreen allergy. The addition of MI to an allergen screening series in the United States and Europe will likely uncover otherwise undiagnosed cases of preservative contact allergy. Likely if we look (and test), we will find a surprising and important prevalence of MI contact allergy. Only then will we understand the extent of the problem, and perhaps, have some data to suggest something further be done about it.[31]

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EXHIBIT H

SCCS/1521/13
Revision of 27 March 2014

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Methylisothiazolinone (P94)

Submission II

(Sensitisation only)

The SCCS adopted this opinion at its 4th plenary meeting

on 12 December 2013

Revision of the opinion on methylisothiazolinone (P94)

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS
The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members
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The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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This opinion has been subject to a commenting period of eight weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

Keywords: SCCS, scientific opinion, cosmetic ingredients, methylisothiazolinone, MI, Regulation 1223/2009, contact allergy, epidemic, CAS no. 2682-20-4, EC 220-239-6

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Methylisothiazolinone (P94) – Submission II, 12 December 2013, SCCS/1521/13, revision of 27 March 2014.

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Revision of the opinion on methylisothiazolinone (P94)

1. BACKGROUND

The Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) adopted the first opinion in March 2003 on "Methylisothiazolinone" (SCCNFP/0625/02).

The SCCNFP adopted the second opinion on "Methylisothiazolinone" on April 2004 (SCCNFP/0805/04) with the following conclusion:

The SCCNFP is of the opinion that the proposed use of Methylisothiazolinone as a preservative at a maximum concentration of 0.01% (100 ppm) in the finished cosmetic product does not pose a risk to the health of the consumer.

Methylisothiazolinone (MI) has been listed in Annex V/57 of the Cosmetic Regulation 1223/2009/ECC to be used as preservative at maximum concentration of 0.01% (100ppm) in cosmetics products.

In the Cosmetic Regulation 1223/2009/ECC Annex V/39 we have also the mixture of Methylchloroisothiazolinone(MCI) and Methylisothiazolinone (MI) that is currently allowed as a preservative in all cosmetic products at a maximum concentration of 0.0015 % (15ppm) of a mixture in the ratio 3:1 of the two substances.

Several Member States raised concern on the use of Methylisothiazolinone (MI) as data demonstrates that MI is a sensitizer in animals and a contact allergen in human. The Commission received information on the issue of sensitising potential of MI starting from 2011. According to this information both MCI/MI and MI alone are used in cosmetics and body care products as well as in household products and industrial products, i.e. occupational contactants. However, for a number of years, MI is also increasingly being used alone, without MCI, or in combination with other biocides. Sensitisation to MI is becoming an increasing problem all over Europe, particularly with sensitisation in young children from moist toilet tissue/hygiene moist tissues or cosmetics and Several Member States have asked the Commission to request to SCCS a reassessment of the safety of the MI when it is used as preservative in cosmetics products at maximum concentration of 100ppm.

2. TERMS OF REFERENCE

1. *On the basis of the new evidence in relation to sensitising potential, does the SCCS consider Methylisothiazolinone (MI) still safe for consumers, when used as a preservative in cosmetic products up to concentration limit of 100ppm? If no, it is asked for the SCCS to revise this concentration limit on the basis of information provided.*

2. *Does the SCCS have any further scientific concerns with regard to the use of Methylisothiazolinone (MI) in cosmetic products?*

3. OPINION

3.1. Chemical identity

3.1.1. Primary name and/or INCI name

INCI methylisothiazolinone

3.1.2. Chemical names

Methylisothiazolinone

IUPAC 2-Methylisothiazol-3(2H)-one

Other 2-Methyl-4-isothiazolin-3-one

3.1.3. Trade names and abbreviations

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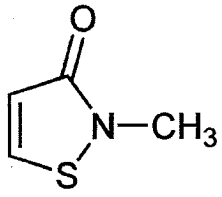
3.1.4. CAS / EC number

CAS no. 2682-20-4

EC 220-239-6

3.1.5. Structural formula

Methylisothiazolinone

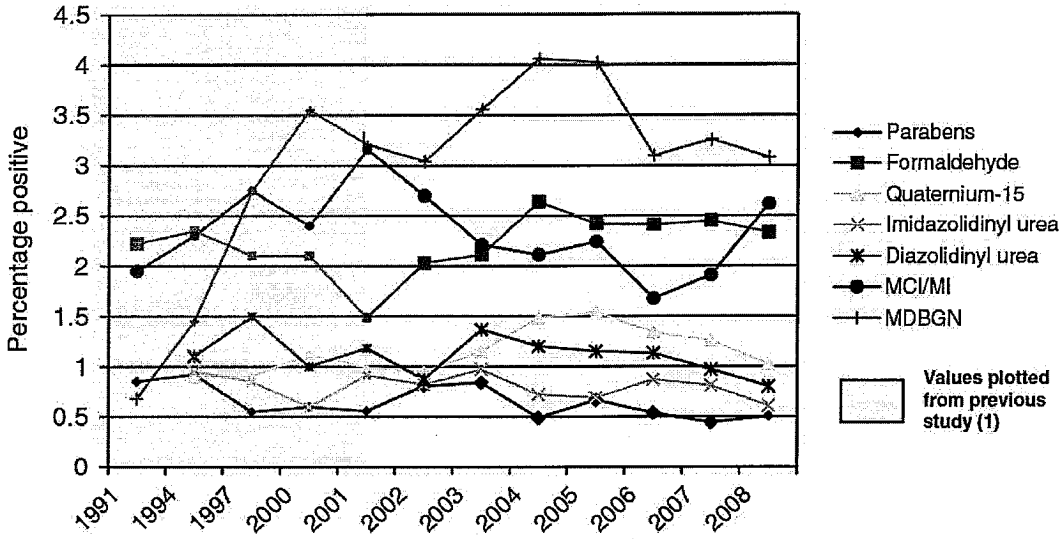


3.2. Epidemiology of Contact Allergy of Methylisothiazolinone

Normal exposures to preservative-containing products by the consumer can induce contact allergy, the elicitation phase of which manifests itself as allergic contact dermatitis.

Several preservatives are included in the European baseline series of contact allergens, used for diagnostic patch test investigations of individuals with eczema. Included in the series is methylchloroisothiazolinone and

methylisothiazolinone (MCI/MI in a 3:1 mixture). Data are available and published at intervals by dermatologists to monitor the trends in contact allergy to preservatives in Europe over time. Such recent pan-European data are illustrated below (Svedman C, Andersen KE, Brandão FM, *et al.* 2012):



For MCI/MI, stepwise risk management measures were introduced and following "safe" limits of this preservative in cosmetics in the EU (15 ppm), contact allergy to MCI/MI significantly decreased to around 2% of patch tested patients after the 90's.

MI was reported to be a weak sensitizer in the guinea pig (Bruze M, Fregert S, *et al.* 1987) but categorised as a strong sensitizer in the local lymph node assay with an EC3 of 0.4% in acetone: olive oil (AOO, see table from Basketter D A, Gilmour N J, Wright Z M, *et al.* 2003).

EC3 values (% v/v)

Vehicle	AOO	PG
Formaldehyde	0.4	2.8
Glutaraldehyde	0.07	1.5
MCI/MI	0.0082	0.063
MI	0.4	2.2

This latter information was not available for inclusion in the SCCNFP Opinion of March 2003 (SCCNFP/0625/02) or the updated opinion of April 2004 (SCCNFP/0805/04) and no LLNA information was included in these opinions. The sensitisation studies made available and considered by the SCCNFP for their Opinions are included within the present Opinion together with a review of the LLNA mentioned above.

The primary sensitising properties of MCI/MI have been attributed to MCI whereas MI was considered unable to sensitize individuals in concentrations below 1000 ppm (Burnett, CL, Bergfeld WF, Belsito DV *et al.* 2010).

MI alone (without MCI) was introduced as a preservative in industrial products in the early 2000's, and in 2005 was allowed as a preservative in both leave-on and rinse-off cosmetics at a maximum concentration of 100 ppm (0.01%) (Annex V/57 of the Cosmetic Regulation 1223/2009/ECC; Cosmetic Directive 2005/42/EC) with an increasing use since (Lundov M D, Krongaard T, Menné T L, Johansen J D. 2011; Castanedo-Tardana M P, Zug K A. 2013).

The first reports on contact allergy from MI appeared in 1987 (Bruze M, Dahlquist I, Fregert S, *et al.* 1987). After 2000, MI was introduced in industrial products (e.g., paints, adhesives, varnishes and cooling fluids), and due to its weaker preservative effect was used at higher concentrations than in MCI/MI. Allergic contact dermatitis from MI in the occupational setting was reported in 2004 (Isaksson M, Gruvberger B, Bruze M. 2004). Several cases of occupational allergic contact dermatitis from MI were then reported from paints (Thyssen, J. P., Sederberg-Olsen, N., Thomsen, J. F. & Menné, T.2006; Mose, A. P. *et al.* 2012).

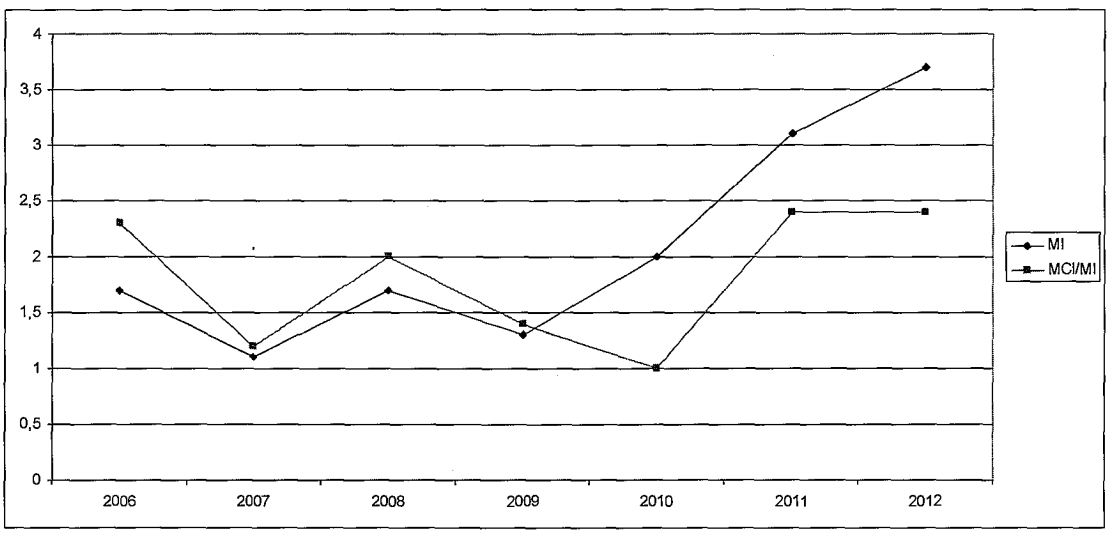
The first reports from cosmetics began in 2010 (García-Gavín J, Vansina S, Kerre S, *et al.* 2010) mainly due to wet wipes for hygiene (baby wipes, moist tissues, moist toilet paper), hair cosmetics (shampoos), facial cosmetics (Lundov, M. D., Thyssen, J. P., Zachariae, C. & Johansen, J. D. 2010; Lundov, M. D., Krongaard, T., Menné, T. L. & Johansen, J. D. 2011) , deodorants (Amaro, C., Santos, R. & Cardoso, J. 2011) and sunscreens (Castanedo-Tardana, M. & Zug, K.2013).

Airborne exposure to MI has caused severe cases of airborne allergic contact dermatitis and systemic contact dermatitis particularly from recently painted walls (Aerts, O. *et al.* 2013; Lundov M. D., Zachariae, C., Menné, T. & Johansen, J. D. 2012) or from toilet cleaners (Lundov, M. D. & Menné, T. 2013), including a case in a 4-year-old child most probably sensitized to MI through baby wipes (Aerts, O. *et al.* 2013).

Accompanying the increasing number of published cases of allergic contact dermatitis from MI, particularly since 2009, a rise in contact allergy to MCI/MI has been observed in Europe.

In Germany, with more than 12 000 patients tested/year, positive patch tests to MCI/MI increased from 2.3% in 2009 to 3.9% in 2011 (Geier J, Lessmann H, Schnuch A, Uter W. 2012) similar to Leeds, UK (increase from 0.9 to 4.9%) (Urwin, R. & Wilkinson, M. 2013) and in Amersham, UK (from < 3% to >8%) (Orton D & Willis C. 2013). In Coimbra, Portugal, reactivity to MCI/MI rose from 1.5% (in 2006/7) to 2.9 and 3.6% respectively in 2011 and 2012 (Gonçalo M, Goossens A. 2013). Similar figures are being observed elsewhere in Europe, with alerts particularly during late 2012 in France and Belgium at REVIDAL, a system to collect alerts in contact dermatitis (Hosteing S, Meyer, N; Waton, J. *et al.* 2013).

The rise in contact allergy to MCI/MI cannot be explained by a change in exposure to MCI/MI in cosmetics (the permitted maximum concentration has been 15 ppm since February 1989 (89/174/EEC), and phasing out of its use in leave-on cosmetic products may enter into force in 2014, see below), but is due to the increasing exposure to MI, present in concentrations very near 100 ppm both in leave-on and rinse-off cosmetics, as illustrated from Denmark (Lundov M D, Krongaard T, Menné T L, Johansen J D. 2011). Further, there is some indication of the levelling-off of the frequency of reactions to MCI/MI whilst MI continues to increase (Lundov M D, Morten S, Opstrup MS, Johansen J D. 2013).



Prevalence of methylisothiazolinone (MI) and methylchloroisothiazolinone (MCI)/MI patch test positive patients at Gentofte Hospital from 2006 to 2012. (Lundov M D, Mortsen S, Opstrup M S, Johansen J D. 2013)

MI has only recently been tested as a single allergen, separate from MCI/MI in the European baseline series, in the local baseline series in several countries. Reactivity was around 1.5% until 2008 in Denmark (Lundov, M. D., Krongaard, T., Menné, T. L. & Johansen, J. D. 2011) but values increased from 0.9% in 2006 to 1.8% in 2008 in Finland (Ackermann, L. *et al.* 2011) and very high values were detected in 2011/12 in Leeds (4.6%) (Urwin, R. & Wilkinson, M. 2013), London (6%), Coimbra (4.5%) and Leuven, Belgium (5.8%), with a very high percentage of relevant reactions (Gonçalo M, Goossens A. 2013).

In Germany, although in selected patients with suspected cosmetic or occupational exposure, MI reactivity rose from 1.9% in 2009 to 4.4% in 2011, particularly in female patients (188% increase) and in patients with facial dermatitis (200% increase), suggesting that increase in reactivity is most probably related to cosmetic exposure (Geier, J., Lessmann, H., Schnuch, A. & Uter, W. 2012). In the USA a similar situation seems to have occurred as MI was considered the allergen of the year 2013 (Castanedo-Tardana, M. & Zug, K. 2013).

Testing with standard patch test preparations of MCI/MI contain too low a concentration of MI to properly demonstrate contact allergy to MI. A patch test concentration of 300 ppm MCI/MI fails to detect almost half of the cases of contact allergy to MI. Increasing to 1000 ppm MCI/MI is not irritating and detects more and relevant cases with subjects reacting in the repeated open application test (ROAT) with a cream containing 100 ppm MI, the highest allowed concentration in cosmetics (Ackermann, L. *et al.* 2011). MI has now been recommended to be included in the European baseline patch test series and tested at 2000 ppm (0.2%). (Bruze M, Engfeldt M, Gonçalo M and Goossens A. 2013).

Contact allergy to MI has been reported in consecutively tested dermatitis patients in Sweden, Denmark, Germany, Finland, and the UK. The contact allergy rates reported vary between 0.5% and 6% in 2012. The highest rates were from the UK, where an increase was noticed in Amersham from 2.5% in 2009 to 6.0% in 2011 (Orton D, Willis C. 2013) and in Leeds from 0.6% in 2009 to 4.6% in 2012 (Urwin R, Wilkinson M. 2013). In Denmark an increase from 1.4% in 2009 to 3.1% in 2011 was recorded (Lundov M D, Zachariae C, Menné T, Johansen J D. 2012). Aimed testing of MI has been performed in Germany where MI has been tested at 500 ppm in water respectively (Geier J, Lessmann H, Schnuch A, Uter W. 2012; Schnuch A, Lessmann H, Geier J, Uter W. 2011). This testing has identified an increasing contact allergy rate to MI from 0.5% before 2009 to 4.4% in 2011.

A male predominance has been reported from the UK (Orton D, Willis C. 2013), Denmark (Lundov M D, Thyssen J P, Zachariae C, Johansen J D.

2010), Germany (Schnuch A, Lessmann H, Geier J, Uter W. 2011), and Sweden (Isaksson M, Gruvberger B, Bruze M. 2013).

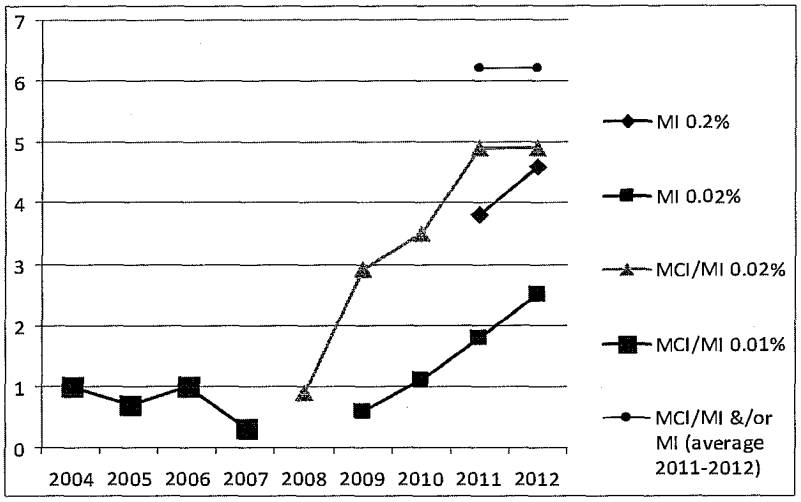
The table below (adapted from: Bruze M, Engfeldt M, Gonçalo M and Goossens A. 2013) shows data on patch test preparations used and contact allergy rates to MI in various European publications. The data above the thick line concerns consecutively tested dermatitis patients, while the information below the thick line concerns aimed testing of groups of patients.

Member State	Conc. in ppm (all aq. except when stated)	Dose in µg/cm^2	Number tested	MI allergy rate %	% of MI positive, MCI/MI negative	Years	Ref
Germany	500	NA	2167	0.8	0.2	2005	A
Denmark	2000	60***	2536	1.5	0.8	2006-2010	D
Finland	300	NA	10 821	0.6		2006-2008	E
	1000	NA	10 821	1.4	0.5	2006-2008	E
UK	500	NA	1337	4.0(2.5-6)	0.1*	2009-2011	B
	200	NA	349	0.6	NA	2009	C
	200	NA	771	1.1	NA	2010	C
	200	NA	611	1.8	NA	2011	C
	200	NA	325	2.5	NA	2012	C
	2000	NA	238	3.8	1.6**	2011	C
	2000	NA	325	4.6	1.6**	2012	C
Sweden	475	14.3	100	1.0	0*	2003	G
	950	28.5	1457	0.7	0	2003-2005	G
	1000	30.0	181	0.5	0	2005	G
Germany	500 pet.	NA	13 433	1.5	0.5	(2005) 2008-2009	F
	500	NA	6789	1.9	1.2	2009	A
	500	NA	7193	3.4	1.2	2010	A
	500	NA	7292	4.4	1.2	2011	A

NA = not available
* = MCI/MI tested at 200 ppm
** Figure is only given for 2011 and 2012 together. MCI/MI was tested at 200 ppm.
*** unpublished, JD Johansen (Denmark)

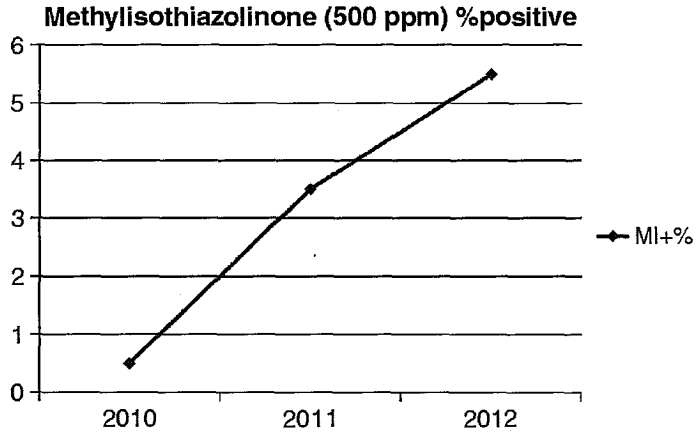
A. Geier J, Lessmann H, Schnuch A, Uter W. 2012
B. Orton D , Willis C. 2013
C. Urwin R, Wilkinson M. 2013
D. Lundov M D, Thyssen J P, Zachariae C, Johansen J D. 2010
E. Ackermann L, *et al.* 2011
F. Schnuch A, Lessmann H, Geier J, Uter W. 2011
G. Isaksson M, Gruvberger B, Bruze M. 2013

The graph below illustrates the rising frequency of contact allergy to MI in a single centre in the UK (Leeds) (Urwin R, Wilkinson M. 2013):

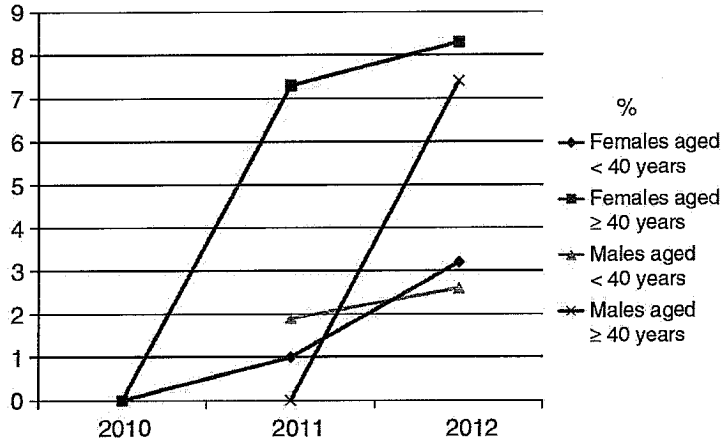


Percentage (y-axis) patch test sensitivity at day 4 (D4) to methylisothiazolinone (MI) and methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) mix at The Leeds Centre for Dermatology January 2004 - June 2012

The graph below illustrates the rising frequency of contact allergy to MI in a further single centre in the UK (London) with consecutive testing of patients with 500 ppm MI (McFadden JP, Mann J, White JML, Banerjee P, White IR. 2013):

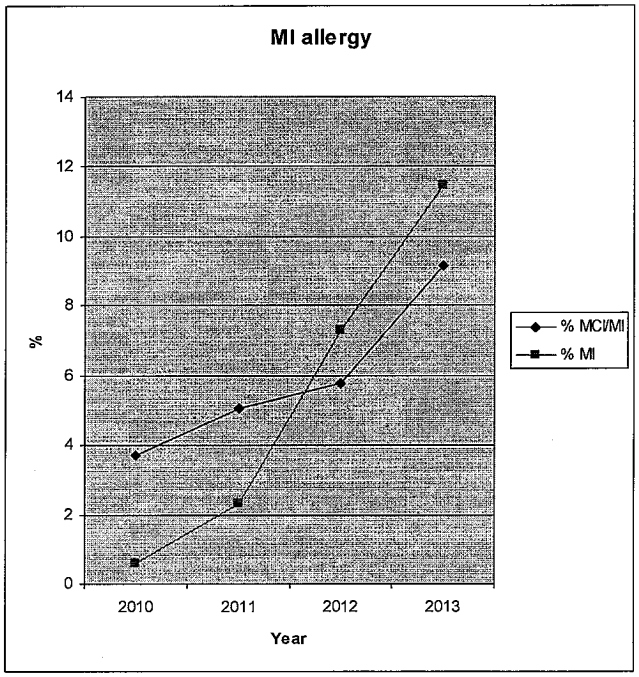


The same data extracted for age and sex shows that males and females ≥ 40 years old were the first to be sensitised in the recent emergence of MI as an important allergen. Possible immuno-mechanistic reasons for this observation have been discussed. (McFadden JP, White IR, Basketter D, Puangpet P and Kimber I. 2013).



Methylisothiazolinone allergy by age and sex. (McFadden JP, Mann J, White JML, Banerjee P, White IR. 2013)

The trend is further illustrated by multicentre data from the British Society for Cutaneous Allergy (BSCA) (Johnson G 2014; reproduced with permission from the author), which includes available 2013 data from contributing centres in the British Isles (UK and Republic of Ireland).



(Johnson G. 2014, combined multicentre date from British Isles). Percentage of patients having contact allergy to MI.

Detailed data from the IDVK in Germany also illustrates the rise in the frequency of MI sensitisation reported from their contributing centres up to 2012 (Uter W, Geier J, Bauer A, and Schnuch A. 2013).

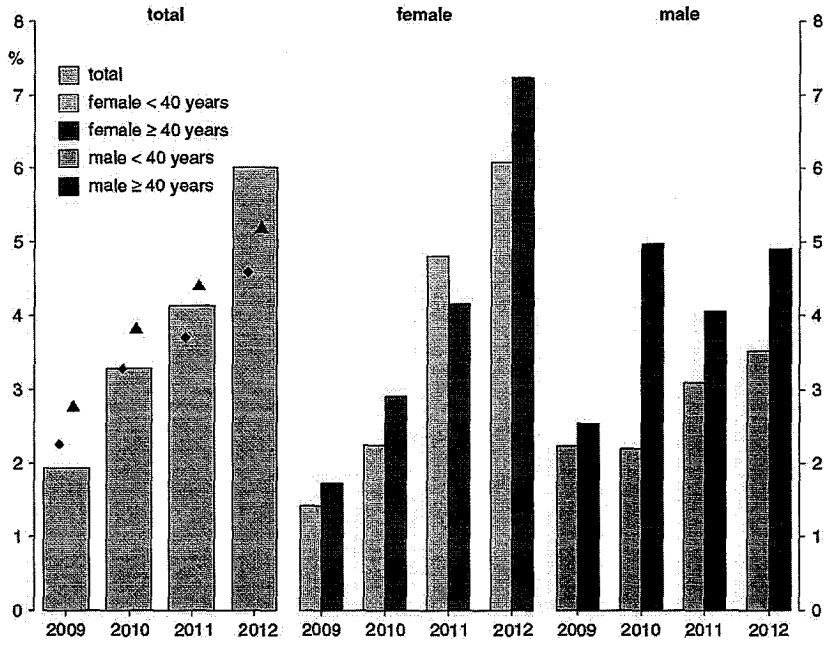


Fig. 1. Annual proportions of positive reactions to methylisothiazolinone (MI), 500 ppm aqua, between 2009 and 2012 in departments of the IVDK. Diamonds: the prevalence of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) (100 ppm aqua, tested in the baseline series) sensitization among consecutively tested patients. Triangles: the results with MCI/MI in patients tested with the cosmetic preservatives series [see also (3)].

These IVDK authors observed a strong association between female sex and face dermatitis, resulting from the use of various cosmetic products.

A significant association was also found with hand eczema as well as face dermatitis. Ano-genital dermatitis was observed in 2.8% of the patients emphasizing the importance of intimate hygiene wipes as a source of exposure to MI. (Uter W, Geier J, Bauer A, and Schnuch A. 2013).

SCCS Comment

Recent and current clinical data, from widely distributed geographical areas within Europe, demonstrate a rapidly increasing frequency of contact allergy to MI.

Below (with typographical errors in the original corrected) are the relevant MI sensitisation report summaries reproduced from SCCNFP Opinion (SCCNFP/0625/02) which suggested that MI 100 ppm to be safe in both leave-on and rinse-off cosmetic products. The reference numbers are those used in the original Opinion (SCCNFP/0625/02) but the full references are

included below each reference number. There is no attempt to re-interpret the data or otherwise comment on the conclusion of the Opinion.

3.3 Irritation and corrosivity

3.3.1. Skin irritation

From SCCNFP 0625/02

2-Methyl-4-isothiazolin-3-one was applied undiluted by a single application of 0.5 ml to the shaved intact skin of 7 New Zealand White rabbits. Contact time was 3 minutes for 5 animals, 1- hr (and 3 min.) for one animal, 4-hrs for one animal. The application sites were semi-occluded. After removal of the patch, the animals were observed for 14 days for signs of irritation.
No mortality or clinical signs of systemic toxicity were observed during the study.
On the 4 and 1 hr sites, skin irritation indicative of corrosivity was observed on day 14 and 7, respectively.
On the 3 min. site, very slight to well defined erythema was noted through day 7 and slight oedema was noted at 1 hr.
For the 5 animals exposed during 3 min., very slight to well defined erythema was noted through 48 hrs in most rabbits. Very slight to moderate oedema was noted at 1 and 24 hrs. Very slight to slight oedema was noted in one rabbit at 48 and 72 hrs.

2-Methyl-4-isothiazolin-3-one is corrosive to the skin when applied undiluted.

Ref.: 5 (SCCNFP 0625/02)

Rohm and Haas Report No 96R-123, RH-573T (undated)- Skin Irritation Study in Rabbits

0.5 ml of an aqueous solution of Neolone™ 950 (100 ppm a.i. [MI] was applied to the skin of a group of six New Zealand White rabbits. Contact time was 4 hours and the application was semi occluded. After removal of the patch, the animals were observed for 72 hours for signs of irritation.

No mortality or clinical signs of systemic toxicity were observed during the study. There was no erythema or oedema present at any observation period. The Primary Irritation Index (PH) was 0.

An aqueous solution of MI is non-irritating to rabbit skin at proposed recommended use concentrations of 100 ppm active ingredient.

Ref.: 6 (SCCNFP 0625/02)

Rohm and Haas Report No OOR-006 (2000) Neolone™ 950 Preservative (100 ppm aqueous solution) Skin Irritation Study in Rabbits.

Human study (modified HRIPT)

The cumulative irritation potential of 2-Methyl-4-isothiazolin-3-one (RH-573; MI) was - investigated in a 21 day test with human volunteers. Aqueous dilutions of MI (0.1 ml) were applied to the back under occlusive patches, for a contact period of 23 hours on 21 consecutive days. On completion of the dosing phase, the subjects were rested without further dosing for 10 - 14 days. Following the rest period, 24 hour patch(es) of the appropriate test material(s) were applied to a naive site. Subject induced with 50, 100 and 250 ppm MI were challenged with the same respective concentrations of test material as well as distilled water and sodium lauryl sulphate. Subjects induced with 500 ppm MI were challenged with 100,250 and 500 ppm MI as well as with distilled water and SLS. Four of the subjects induced with 1000 ppm were not only challenged with 1000 ppm but also with 250 and 500 ppm.

Group	Number of subjects	Introduction concentration of MI (ppm)	Total Reactions during dosing	Cumulative irritation	Challenge concentration of MI (ppm)	Reactions on challenge
I	16	50	11/16	0/16	50	0/16
11	15	100	4/15	0/15	100	0/15
III	17	250	6/17	0/17	250	0/17
IV	15	500	7/15	0/15	500	1/15
					250	1/15
					100	0/15
V	16	1000	15/16	1/16	1000	2/16
					500	1/4
					250	1/4

During the introduction phase, a number of irritant reactions (to both MI and vehicle control - distilled water) were observed, these were mainly graded as 1 and were transient in nature. The total reactions for vehicle controls were 7/16, 4/15, 4/17, 4/15 and 12/16 for the groups I, II, III, IV and V respectively. No reactions were noted on challenge for the vehicle controls in any group.

Cumulative irritation was only observed in one individual from the 1000 ppm induction group.

Ref: 8 (SCCNFP 0625/02)

Rohm and Haas Report No. 92RC-097A. (1994) RH-573 - Evaluation of 21-Day Cumulative Irritation Potential in Humans

3.3.2. Mucous membrane irritation

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3.4 Skin sensitisation

The skin sensitisation potential of 2-Methyl-4-isothiazolin-3-one [MI; RH-24,573] was determined using the closed patch method of Buehler [OECD 406]. Four groups of outbred Hartley guinea pigs [5/sex/group] received ten 6-hr induction doses [3 doses/week for 3.5 weeks] of 0.4 ml at concentrations of 1000, 5000, 15,000 and 30,000 ppm a.i. in distilled water. Two weeks after the last induction dose these animals, together with a group of uninduced control animals, were challenged with 1000, 5000, or 15,000 ppm MI in distilled water.

No erythema reactions were observed in the non-induced control animals at any challenge concentration of MI.

	Challenge dose (ppm a.i.)		
Induction dose **[ppm** a.i.]	**1000**	**5000**	**15000**
0			
1000	0/10	0/10	1/10
5000	0/10	2/10	6/10
15000	1/10	1/10	3/10
30000	0/10	2/10	5/10

The concentration of MI required to induce and elicit a response in 50 % of the animals (EC_{50}) was estimated to be ≥ 5000 ppm a.i. for induction at a challenge concentration 15,000 ppm a.i. and ≥ 15,000 ppm a.i. for challenge at an induction concentration of 30,000 ppm a.i.

Ref 9: SCCNFP 0625/02

Rohm and Haas Report No. 88R-052. (1989) RH-24,573: Delayed Contact Hypersensitivity Study in Guinea Pigs.

Human Repeated Insult Patch test

One subject, from the 500 ppm induction group (see table above), was found to react on challenge. This individual was found to react to the marker pen and also to a number of consumer products; this reaction was therefore considered to equivocal. Two subjects from the 1000 ppm

induction group showed a mild reaction upon challenge and were considered to be sensitised.

Based on this data the threshold for sensitisation appears to be at around 1000 ppm 2-Methyl- 4-isothiazolin-3-one.

Re: 8 SCCNFP 0625/02

Rohm and Haas Report No. 92RC-097A. (1994) RH-573 - Evaluation of 21-Day Cumulative Irritation Potential in Humans

In an intensified Shelanski and Shelanski Repeated Insult Patch Test, ninety-eight [98] adult volunteers, patch test negative to 100 ppm Kathon® CG, were enrolled into the study. Kordek™ 50C (Methyl-4-isothiazolin-3-one; MI), 0.15 ml of a 100 ppm aqueous solution, was applied to a webril pad and the pad applied to the back of the volunteers under occlusion. Patches were applied four times a week for three weeks [induction phase]. After a week free of dosing, the subjects were challenged on a fresh site with MI, 0.15 ml of a 100 ppm aqueous solution applied to a webril pad. One of the 98 subjects showed a positive response [grade 4] on the fifth day of the induction phase. This subject was judged to be pre-sensitised. Of the remaining 97 subjects none reacted to challenge [elicitation] with 100 ppm aqueous MI.
Under the conditions of this test, 100 ppm Methyl-4-isothiazolin-3-one did not induce skin sensitisation in human volunteers.

Ref: 10 SCCNFP 0625/02

Rohm and Haas Report No. 99RC-0138. (2000) A Patch Test to Determine the Skin Irritation and Sensitisation Propensities of Kordek TM 50C (study conducted at 100 ppm active ingredient)

In a Repeat Insult Patch Test, 113 adult volunteers (12 males and 101 females), were enrolled in the study. 0.2 ml of an aqueous solution of 200 ppm 2-Methyl-4-isothiazolin-3-one, was applied by occlusive patches for a contact period of 24-hr per day. Patches were applied three times a week for three weeks [induction phase]. After a week free of dosing, the subjects were challenged on a fresh site with MI, 0.2 ml of a 200 ppm aqueous solution applied to a webril pad.

There was no adverse effect reported in the 100 subjects who completed the study. 13 out of 113 enrolled in the study either violated the protocol or withdrew from the study.

Under the conditions of this test, 200 ppm Methyl-4-isothiazolin-3-one did not induce skin sensitisation in human volunteers.

Ref: 11 SCCNFP 0625/02

Rohm and Haas Report No. 00RC-099A. (2000) Repeat Insult Patch Study with 2-methylisothiazolin-3-one at an Aqueous Concentration of 200 ppm Active Ingredient.

In a Repeat Insult Patch Test, 107 adult volunteers (19 males and 88 females), were enrolled in the study. 0.2ml of an aqueous solution of 300 ppm 2-Methyl-4-isothiazolin-3-one, was applied by occlusive patches for a contact period of 24-hr per day. Patches were applied three times a week for three weeks [induction phase]. After a week free of dosing, the subjects were challenged on a fresh site with MI, 0.2 ml of a 300 ppm aqueous solution applied to a webril pad. There was no adverse effect reported in the 98 subjects who completed the study. 9 out of 107 enrolled in the study either violated the protocol or withdrew from the study.

Under the conditions of this test, 300 ppm Methyl-4-isothiazolin-3-one did not induce skin sensitisation in human volunteers.

Ref: 12 SCCNFP 0625/02

Rohm and Haas Report No. OORC-099B. (2000) Repeat Insult Patch Study with 2-methylisothiazolin-3-one at an Aqueous Concentration of 300 ppm cove Ingredient.

Sensitisation Potency of MI in relation to MCI/MI

Animal Data

In a study using the Buehler method (Ref. 9 (*Rohm and Haas Report No. 88R-052. (1989) RH-24,573: Delayed Contact Hypersensitivity Study in Guinea Pigs*)), the concentrations of RH-24,573 [MI] required to induce and elicit a response in 50% of guinea pigs [EC_{50}] (the effective concentration producing the effect under study in 50% of the test population) were estimated. The EC50 for induction was determined to be > 5000 ppm a.i. at a challenge concentration of 15,000 ppm a.i. For elicitation, the EC_{50} was 15,000 ppm a.i. at an induction concentration of 30,000 ppm a.i. A similar study (Ref. 28 (*Chan, P. K., Baldwin, R. C., Parsons, R. D., Moss, J. N., Stiratelli, R., Smith, J. M. and Hayes, A. W. [1983] Kathon Biocide: Manifestation of Delayed Contact Dermatitis in Guinea Pigs is Dependent on the Concentration for Induction and Challenge. Journ.Investigat. Dermatol: 81: 409 - 411*)) performed with MCI/MI [3/1] determined the EC_{50} for induction to be 88 ppm a.i. at a challenge concentration of 2,000 ppm a.i., and the EC_{50} for elicitation to be 429 ppm a.i. at an induction concentration of 1,000 ppm a.i.

In a version of the Local Lymph Node Assay (Ref. 29 (*Potter, D. W. and Hazelton, G. A. [1995]. Evaluation of Auricular Lymph Node Cell Proliferation in Isothiazolone-Treated Mice. Fundamental and Applied Toxicology 24 165- 172*)), the PC_{200} [the concentration giving a 2-fold proliferate response over controls] for MI was 1506 µg and for MCI was 11 µg.

The sensitisation potential of methylchloroisothiazolinone

/methylisothiazolinone [3: 1] was compared to that of Methylisothiazolinone in the Open Epicutaneous test. The threshold for induction for MCI/MI was 58 ppm (Ref. 30 (*Wiemann, C. and Hellwig, J. [2001]. Methylisothiazolinone 20% - Open Epicutaneous Test in Guinea Pigs*)). For MI the induction threshold was in the range of 3000 ppm. (Ref. 31 (*Wiemann, C. and Hellwig, J. [2001].]. Chloromethylisothiazolinone/Methylisothiazolinone 3:1 Open Epicutaneous Test in Guinea Pigs*))

Human Data

Although no comparative Human Repeat Insult Patch tests have been performed on MCI/MI and MI, it is possible to compare the sensitisation potential based on existing studies of the two substances. The available data, taken partly from Company reports and partly from the open literature, is summarised in the table below.

Comparison of Human Repeat Insult Patch Test data on MCI/MI and MI

	MCI/MI [3:1]			MI		
Concentration [ppm]	Dose [µg/cm^2]	Incidence	% Response	Dose [µg/cm^2]	Incidence	Response
	0.42	0/416	0.0	-	-	-
	0.50	0/103	0.0	-	-	-
7.5	0.75	0/184	0.0	-	-	-
10	0.83	0/602	0.0	-	-	-
12.5	1.04	1/84	1.2	-	-	-
15	1.25	0/200	0.0	-	-	-
15	1.34	2/189	1.1	-	-	-
20	1.67	2/45	4.4	-	-	-
50[b]	2.50	0/109	0.0	-	-	-
100[b]	5.00	5/1 16	4.3	5	0/97	0.0
150[c]	7.50	7/196	3.6	-	-	-
200	-	-	-	10	0/100	0.0
300	-	-	-	15	0/98	0.0
400	-	-	-	20	1/116	0.9
500	-	-	-	45	1/210	0.5
600[d]	-	-	-	30	0/75	0.0

b: Based on the summation of results of Draize tests conducted by Maibach cited in ref.6 p. 105
c: Subjects received six induction exposures at 150 ppm in petrolatum followed by four induction exposures at 300 ppm in water - Maibach cited in ref. 6 p 104
d: Study in progress; the results to date are reported.

Ref: 32, 33, 34 SCCNFP 0625/02

Cosmetic Ingredient Review (CIR) Expert Panel: Final Report on the Safety Assessment of Methylisothiazolinone and Methylchlororisothiazolinone, J. Amer. College Toxicol., Vol. 11 (1), 1992.

Fewings, J. and Menne, T.: An Update of the Risk Assessment for methylchloroisothiazolinone/ methylisothiazolinone (MCI/MI) with Focus on Rinse-Off Products, Contact Dermatits, Vol. 41, 1999.

Repeat Insult Patch Studies with 2-Methylisothiazolin-3-one at Aqueous Concentrations of 100 to 600 ppm Active Ingredient : Rohm and Haas Report Numbers: 99RC-01 38, OORC -099A, OORC-099B, OORC-099C, OORC099D, OORC-099E, OORC-099F; conducted:1999-2001.

Based on the results of the HRIPT data, there is at least a factor of 30 difference in the sensitisation [induction] potential of the two isothiazolinone products. This compares favourably with the Open Epicutaneous Test [OET] which shows a factor of 50 difference in sensitisation [induction] potential. Thus, on the basis of this data, the number of new sensitisations induced by exposure of people to MI through the use of cosmetic and toiletry products is predicted to be low.

Dose-elicitation studies of Methylisothiazolinone on individuals known to be allergic to Kathon® CG

In a study, 28 patients sensitised to MCI/MI were patch tested with MCI and MI and all individuals reacted to MCI at 300 ppm, whereas only 2 reacted to MI at 300 ppm [one also reacted to 100 ppm MI].

Ref: 35 SCCNFP 0625/02

Bruze, M., Dahlquist, I., Fregert, S., Gruveberger, B. and Persson, K. Contact allergy to the active ingredients of Kathon® CG. Contact Dermatitis 1987: 16: 183 - 188

Further studies showed that of 12 subjects previously sensitised to MCI/MI [all reacted to a 150 ppm patch of MCI/MI] 3 reacted to MI at 115 ppm with weak reactions, recorded by the authors as 'doubtful'.

Ref: 36 SCCNFP 0625/02

Bruze, M., Dahlquist, I. And Gruvberger, B. [1989] Contact allergy to dichlorinated methylisothiazolinone. Contact dermatitis 20: 219 - 239.

In a study, 85 subjects from the clinics of the IVDK identified as patch positive to MCI/MI, were patch tested with MI at concentrations of 500 ppm a.i or 1000 ppm a.i. in water. The allergic status towards MCI/MI was compared with the responses to the MI patches. The results are shown in tables 2 and 3.

Ref: 37 SCCNFP 0625/02

Schnuch, A. [1999] Testing the Frequency of Sensitisation to MI in MCI/MI (Kathon CG) sensitized subjects. Un-published study conducted for Rohm and Haas.

Table 2: Reactions of the MCI/MI-positive test subjects to MI

No. Subjects	Total MI negative	MI negative (500 ppm)	MI positive (500 +1000 ppm)
85	58 [68%]	9 [11 %]	27 [32%]

Table 3: Reactions of the MCI/MI-positive test subjects to MI graded by response to MCI/MI

No. Subjects	MI positive	MCI/MI (+) and MI positive	MCI/MI *(++1+++)* and MI positive	MCI/MI *(++1+++)* and MI negative
85	27 [32%]	12 [14%]	11 [13%]	7 [8%]

In the 73 subjects where the intensity of the MCI/MI reaction was reported, there is a highly significant correlation [$p<0.01$] between the intensity of MCI/MI sensitisation and the reaction to MI. See table 4.

Table 4: Relationship between the intensity of MCI/MI sensitisation and the reaction to MI

	MI (+/+++) positive	MI (+/+++) negative	Total
MCI/MI (++/+++)	11 [61%]	7 [39%]	18
MCI/MI (+) positive	12 [22%]	43 [78%]	55
Total	23	50	73

The results show that, at high concentrations of MI [500 to 1000 ppm], a proportion of the subjects with a known sensitivity to MCI/MI may also react to MI. Thus, from the available data, it cannot be excluded that patients previously sensitised to MCI/MI will react to products containing 100 ppm MI. However, the numbers are expected to be low and will be further reduced by the warning provided through ingredient labelling.

Importantly, based on the HRIPT data, the number of new sensitisations induced by exposure to MI through the use of cosmetic and toiletry products is expected to be low.

Sensitisation potential of degradation products

Degradation of MCI/MI involves opening of the isothiazolinone ring by nucleophilic attack on the ring sulphur. During the nucleophilic attack, the chlorine atom at position 5 of the isothiazolinone ring leaves, thus both MCI and MI will essentially follow the same metabolic/degradation pathways.

Once the ring has opened the electrophilic reactivity and biological action is lost.
This was confirmed by Bruze and Gruvberger who failed to find positive reactions when N- methylmalonamic acid, malonamic acid and malonic acid were tested in 10 MCI/MI sensitive patients. Further, inactivation of MCI/MI with sodium bisulphite destroys the sensitisation potential.

Ref: 38, 39 SCCNFP 0625/02

Bruze, M. and Gruvberger, B. Patch Testing with degradation products of Kathon CG. Contact Dermatitis 1989: 21: 124

Gruvberger, B. and Bruze, M. Can chemical burns and allergic contact dermatitis from higher concentrations of methyl chloroisothiazolinone/methylisothiazolinone be prevented? Am. J. Contact Derm 1998 31: 11-14.

Studies <u>not included or unavailable</u> for the SCCNFP Opinion (0625/02) are below:

Local Lymph Node Assay (LLNA)

Study 1

Guideline:	/
Species/strain:	female CBa/Ca mice
Group size:	4 per test dose
Test substance:	2-methyl-2H-isothiazol-3-one (MI)
Batch:	/
Purity:	19.7%-(the remainder water)
Vehicle:	acetone: olive oil, 4: 1 v/v (AOO) and propylene glycol (PG)
Concentration:	0.049, 0.099, 0.197, 0.493, 0.985 (AOO); 0.99, 1.97, 4,93, 9.85 (PG)
Positive control:	MCI/MI, formaldehyde, glutaraldehyde
GLP:	/
Study period:	2003 or earlier

Dosing solutions were freshly prepared each day immediately before treatment. Test concentrations were selected on the basis of the standard approach adopted for the local lymph node assay.
Groups of four mice received 25 µl, of various concentrations of 2-methyl-2H-isothiazol-3-one (MI) in vehicle or vehicle alone on the dorsum of both ears daily for 3 consecutive days. Five days following the initiation of treatment, all mice were injected intravenously with 250 µl, of 20 μCi ^{3}HTdR in PBS. Five hours later draining auricular lymph nodes were excised and a single cell suspension prepared. The incorporation of ^{3}HTdR was measured

by β-scintillation counting and is displayed in the table above as the mean dpm/node for lymph nodes pooled from each group of four mice and the stimulation index (SI) for two vehicles; AOO acetone: olive oil and PG propylene glycol.

Results

Vehicle/ concentration (%)	dpm/node	SI
AOO	355	
0.049	531	1.5
0.099	521	1.5
0.197	633	1.8
0.493	1348	3.8
0.985*	874	2.5
PG	281	
0.99	527	1.9
1.97	724	2.6
4.93	1978	7.0
9.85	2131	7.6

*It was noted that at 0.985%, the highest concentration of MI in AOO, the SI value was reduced, perhaps reflecting the distinct skin irritation observed at this concentration.

The estimated concentration of the test chemical required to induce an SI of 3 relative to concurrent vehicle-treated controls (the EC3 value) was calculated via linear interpolation of the dose response data.

	EC3 values	
	EC3 value	(% v/v)
Vehicle	AOO	PG
Formaldehyde	0.4	2.8
Glutaraldehyde	0.07	1.5
MCI/MI	0.0082	0.063
MI	0.4	2.2

Conclusion

MI has a similar sensitising potency as formaldehyde.

Ref:
Basketter D A, Gilmour N J, Wright Z M, *et al.* 2003

SCCS Comment
An EC3 value can depend on the vehicle. An EC3 of 0.4 categorises MI as a strong sensitizer. Parallel studies with other well-known skin sensitizers provide confirmation of the relative potency of MI.

Not all experimental information, normally expected when assessing original data, is included in the publication.

Study 2

Rohm & Haas investigated the sensitisation potential of 10.37% MI in Neolone™ 950 using female CBA/J mice in an LLNA. There were 5 mice in each of the 6 dose groups and the positive and negative (acetone/olive oil 4:1) control groups. The mice received 25 µL of topical solution consisting of 0%, 0.15%, 0.45%, 0.76%, 1.35%, 1.57%, or 1.80% MI or positive control on each ear for 3 consecutive days. On day 6 of the study, the mice were injected with 20 µCi of ^{3}H thymidine and killed 5 hours later.

The SIs were determined to be 2.08, 2.40, 2.23, 6.64, 4.73, and 6.62 for the 0.15%, 0.45%, 0.76%, 1.35%, 1.57%, and 1.80% MI dose groups, respectively. It was concluded that MIT is a sensitizer at concentrations greater than 0.76%. The EC3 was calculated to be 0.86%.

Ref:

Rohm & Haas, (2003) Report 06R-l002. Methylisothiazolinone: local lymph node assay (methylisothiazolinone 10.37% active ingredient). Unpublished data submitted by Rohm & Haas and referred to in Burnett, CL, Bergfeld WF, Belsito DV *et al.* (2010)

A review paper (Roberts DW, Patlewicz G, Kern PS, *et al.* 2007), in which the specific data source or date of acquisition is unreferenced, states an EC3 1.9% for MI (vehicle etc. not stated), but which would still categorise MI (#9 in the table below) as a strong sensitizer (SCCP/0919/05) and not as 'moderate' as indicated in the table. The same table gives an EC3 value of 0.009 to MI (source unreferenced) and which is similar to an EC3 0.0082 for MCI/MI (Basketter D A, Gilmour N J, Wright Z M, *et al.* 2003)

#	chemical name	CAS #	LLNA EC3%	potency category	group
1	clotrimazole	23593-75-1	4.8	moderate	
2	potassium dichromate	7778-50-9	0.08	extreme	
3	benzo[*a*]pyrene	50-32-8	0.0009	extreme	pro-SN2 PAH, via oxidation (*9*)
4	7,12-dimethylbenz[*a*]anthracene	57-97-6	0.006	extreme	pro-SN2 PAH, via oxidation (*9*)
5	5-chloro-2-methyl-4-isothiazolin-3-one	26172-55-4	0.009	extreme	compounds for which S_N2-reaction at the S-atom can be proposed (*9*)
6	1-methyl-3-nitro-1-nitrosoguanidine	70-25-7	0.03	extreme	*N*-nitroso derivatives, which act as hard S_N2 or pro-S_N2 electrophiles, discussed in some detail in (*9*)
7	*N*-methyl-*N*-nitrosourea, toxic	684-93-5	0.05	extreme	*N*-nitroso derivatives, which act as hard S_N2 or pro-S_N2 electrophiles, discussed in some detail in (*9*)
8	*N*-ethyl-*N*-nitrosourea	759-73-9	1.1	moderate	*N*-nitroso derivatives, which act as hard S_N2 or pro-S_N2 electrophiles, discussed in some detail in (*9*)
9	2-methyl-2*H*-isothiazol-3-one	2682-20-4	1.9	moderate	compounds for which S_N2-reaction at the S-atom can be proposed (*9*)
10	1,2-benzisothiazolin-3-one (proxel active)	2634-33-5	2.3	moderate	compounds for which S_N2-reaction at the S-atom can be proposed (*9*)
11	tetramethylthiuram disulfide	137-26-8	5.2	moderate	compounds for which S_N2-reaction at the S-atom can be proposed (*9*)
12	1-naphthol	90-15-3	1.3	moderate	we suggest that this acts as a Michael acceptor via its keto-tautomer

Ref:
Roberts DW, Patlewicz G, Kern PS, *et al.* 2007.

SCCS Comment

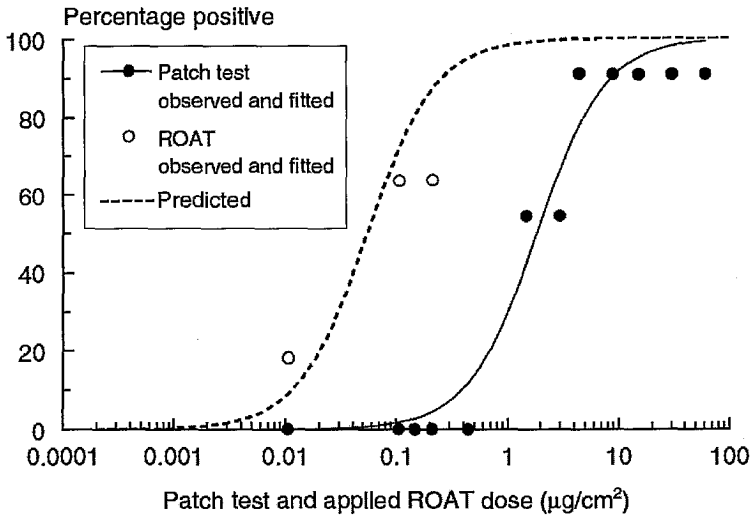
DW Roberts, in a personal communication, recalls that the information was obtained from an earlier paper (Estrada E, Patlewicz G, Chamberlain M *et al.* 2003), in which a so-called 'gold list' of reference EC3 values was published. In this earlier paper the EC3 of MI (item 23 in their list, see below) is given as 1.9; the source of this is unavailable in the paper. In an editorial (Roberts DW 2013) it is indicated that the error can be explained from a failure to take into account the dilution at which MI was tested in the LLNA. However, if one looks elsewhere in the Estrada *et al.* 'gold list', item 22 is given an EC3 0.4. This substance is a hair dye (Colipa A31) for which there is a SCCP Opinion (SCCP/0957/05, March 2006) that it is <u>not</u> a skin sensitiser in a properly performed LLNA.

22	2-methyl-5-hydroxyethylaminophenol	0.4
23	2-methyl-2H-isothiazol-3-one	1.9

Therefore, there appear to be at least two error(s) in the Estrada *et al.* paper. (Estrada, in a personal communication, was unable to comment on the observation). It is unknown whether there are other important errors in the 'gold list' of EC3 values.

The evidence concludes that only one published and properly described LLNA assay has been performed (Basketter D A, Gilmour N J, Wright Z M *et al.* 2003); the EC3 of MI is 0.4%.

Dose-response studies in man



Eleven MI-allergic individuals were patch tested with a dilution series of 12 doses of MI. The lowest eliciting dose in the patch test was 1.47 µgMI/cm^2. (49 ppm).

A repeat open application test (ROAT) mimicked the use of a cream preserved with 100, 50 and 5 ppm MI (corresponding to 0.21, 0.105 and 0.0105 µgMI/cm^2). In the ROAT, 7 patients (64%) reacted to 0.21 and 0.105 µgMI/cm^2 and 2 patients (18%) reacted to 0.0105 µgMI/cm^2, corresponding to a cream preserved with 5 ppm MI.

Ref:
Lundov MD, Zachariae C and Johansen JD. (2011)

SCCS Comment
A relatively small number of subjects were investigated but the study does provide useful information on eliciting-doses of MI in sensitised individuals.

3.5 Discussion

The dramatic rise in the rates of reported cases of contact allergy to MI, as detected by **diagnostic patch tests**, is unprecedented in Europe; there have been repeated warnings about the rise (Gonçalo M, Goossens A. 2013). The increase is primarily caused by increasing consumer exposure to MI from cosmetic products; exposures to MI in household products, paints and in the occupational setting also need to be considered. The delay in re-

evaluation of the safety of MI in cosmetic products is of concern to the SCCS; it has adversely affected consumer safety.

Diagnostic patch tests offer 1) the first indication that exposure to a substance is causing allergy in the population; 2) a means to compare the relative importance of contact allergens in terms of the frequency of reactions and 3) a means of following contact allergy trends over time. (Basketter DA, White IR. 2012)

Diagnostic patch test data do not 1) prove what exposures caused the induction of contact allergy; 2) give any dose-response information or 3) inform on what types of exposure may be tolerated, either for induction or elicitation. (Basketter DA, White IR. 2012)

The elicitation of contact allergy under diagnostic patch test conditions is intended to show whether an individual patient has contact allergy to a substance or not; it is sensitive and specific as a diagnostic tool.

Part of the diagnostic procedure is also an exposure analysis, which provides information about exposures/products, which (may have) initiated the disease manifestation. It is from such analysis that it is known that cosmetic use is associated with MI contact allergy and it may be possible to identify the specific product responsible.

The characteristics of the multiple real life exposures (aggregate exposures) that have led to the induction of contact allergy are rarely well defined. However, they clearly have occurred; for MI, contact allergy has been induced to an alarming extent.

Sensitisation is a relevant toxicological endpoint. An obvious increase in the frequency of sensitisation in the consumer shows that there has been a failure of risk assessment and/or management of the risk.

In the 2009 SCCS Opinion on the mixture of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one (SCCS/1238/09) it was concluded:

> "The mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1 is well recognised as an important skin sensitiser at current conditions of use and applications. Hitherto, it has been used in both leave-on and rinse-off products in Europe.
> Induction and elicitation would be less likely in a rinse-off product than when the same concentration is present in a leave-on product.
>
> "On the basis of the data submitted, the SCCS is of the opinion that the mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1 does not pose a risk to the health of

the consumer when used as a preservative up to a maximum authorised concentration of 0.0015 % in rinse-off cosmetic products, apart from its sensitising potential."

However, despite the mandate on which the above Opinion is based, referring only to rinse-off cosmetic products, there remains no restriction on the use of the mixture (item 39, Annex V, Cosmetics Regulations 1223/2009 with updates) in leave-on cosmetic products. However, it is understood that there is a Commission proposal to restrict the use of MCI/MI in rinse-off products. Meanwhile MCI/MI appears to be now little used in leave-on cosmetic products in Europe.

A separate Opinion on the related preservative benzisothiazolinone (SCCS/1482/12; June 2012) raised concerns about MI:

"As has been seen with MCI/MI and now with MI itself, these isothiazolinones are important contact allergens for the consumer in Europe. Within the mixture, MCI is known to be the more potent allergen (EC3 0.009%). MI is less potent (EC3 1.9%)* and is now permitted at up to 100 ppm in leave-on and rinse-off cosmetic products; contact allergy to MI itself is now a considerable problem in Europe and this is of concern.

(* The EC3 value of 1.9% is incorrect; it is 0.4%. This is discussed above.)

"It is recommended that the incidence of contact allergy to BIT (benzisothiazolinone) and other isothiazolinones be monitored at regular intervals (e.g. annually), by reference to dermatology clinic data in Europe. Necessary early interventions can then be introduced to reduce exposures and hence contact allergy and allergic contact dermatitis as required."

As present in the MCI/MI mixture, the consumer is being exposed to MI at *circa* 3.8 ppm in cosmetic products and this is now supported (by industry but not yet by regulation (SCCS/1238/09) for use only in rinse-off cosmetic products. Up to MI 100 ppm is currently permitted in leave-on (which includes wet wipes) and rinse-off cosmetic products. So, excluding aggregate exposures, the dose of MI per unit area of skin to which the consumer is exposed is *circa* 25x higher than with the MCI/MI mixture.

Leave-on cosmetic products

There is no adequate information to suggest a safe dose of MI in leave-on cosmetic products from the view of *induction* of sensitisation, although *circa* 3.8 ppm, as present in MCI/MI, may be indicative.

The wealth of clinical data demonstrates that 100 ppm MI sensitises.

There is no adequate information as to what doses of MI in leave-on cosmetic products an individual with contact allergy to MI may tolerate, although 5 ppm was not tolerated (*elicitation reactions*) by 2 subjects in the Lundov study (Lundov MD, Zachariae C and Johansen JD. 2011).

Rinse-off cosmetic products

For rinse-off products, it may be considered that *circa* 3.8 ppm MI (as in the MCI/MI mixture) is acceptable as this is the amount present when MCI/MI (3:1) is used at 15ppm for preservation of rinse-off cosmetic products, but it is unknown whether this concentration provides useful preservative activity. (Lundov (Lundov MD 2010) has shown that low concentrations of MI with phenoxyethanol produce active preservation). However, as MCI is a more potent allergen than MI and is the principal moiety in MCI/MI, the SCCS suggests that MI should be safe in rinse-off cosmetic products at 15 ppm (0.0015%). Permitted levels of MI in rinse-off cosmetic products should be safe for previously sensitised individuals but whose allergy has not been shown by formal investigation. Dose-elicitation studies of MI in rinse-off products on individuals with contact allergy to MI are not available.

It may be suggested that Quantitative Risk Assessment (QRA) could be applied to derive safe levels of MI in rinse-off cosmetic products. Although the SCCS considers QRA as a promising tool to prevent induction of contact sensitisation for people with normal skin, the SCCS has insufficient confidence in the model at present (SCCP/1153/08, June 2008).

Ingredient labelling may be used to protect the consumer with a known contact allergy to MI to avoid exposures which may elicit an allergic contact dermatitis. For the assessment of aggregate exposures and the safety evaluation of MI, information on the actual concentrations of MI in consumer products including cosmetics is needed. Since MI is widely used in other consumer products (eg. detergents, paints), exposures from such sources should also be assessed.

It is understood that cosmetic products containing MCI/MI (up to 15 ppm) may not include additional MI.

4. CONCLUSION

1. *On the basis of the new evidence in relation to sensitising potential, does the SCCS consider Methylisothiazolinone (MI) still safe for consumers, when used as a preservative in cosmetic products up to concentration limit of 100 ppm? If no, it is asked for the SCCS to revise this concentration limit on the basis of information provided.*

Current clinical data indicate that 100 ppm MI in cosmetic products is not safe for the consumer.

For leave-on cosmetic products (including 'wet wipes'), no safe concentrations of MI for induction of contact allergy or elicitation have been adequately demonstrated.

For rinse-off cosmetic products, a concentration of 15 ppm (0.0015%) MI is considered safe for the consumer from the view of induction of contact allergy. However, no information is available on elicitation.

2. *Does the SCCS have any further scientific concerns with regard to the use of Methylisothiazolinone (MI) in cosmetic products?*

MI should not be used as an addition to a cosmetic product already containing MCI/MI.

More frequent review of data (than suggested in SCCS/1482/12) to monitor sensitisation frequencies of MI and related isothiazolinone preservatives is recommended. This permits trends in consumers' sensitisation to be observed and timely intervention to be taken.

Information on the actual concentration of MI present in individual cosmetic products will allow future evaluation of safe concentrations.

Labelling is only helpful to a consumer who has a known (established by diagnostic patch test investigations) allergy. It is unknown what proportion of the general population is now sensitized to MI and has not been confirmed as sensitized.

Since MI is widely used in other consumer products (eg. detergents, paints), exposures from such sources should also be assessed.

Consumers cannot find information on the presence of MI in products except in cosmetics and household detergents because, as yet, there is no harmonised classification of MI as a skin sensitizer. The risk for skin sensitisation by MI is at least equivalent to that of other substances which have received a harmonised classification according to the CLP Regulation.

5. MINORITY OPINION

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6. REFERENCES

SCCNFP/0625/02: Evaluation and opinion on: Methylisothiazolinone, 18 March 2003.
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http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_s_01.pdf

SCCP/1153/08: Opinion on dermal sensitisation quantitative risk assessment (citral, farnesol and phenylacetaldehyde), 24 June 2008.
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EXHIBIT I

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Benzisothiazolinone

COLIPA n° P96

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS
The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members
Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Qasim Chaudhry, Gisela Degen, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Jan van Benthem, Jacqueline van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

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The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

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Keywords: SCCS, scientific opinion, preservative, benzisothiazolinone, P96, directive 76/768/ECC, CAS 2634-33-5, EC 220-120-9

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on benzisothiazolinone, 26-27 June 2012

This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

TABLE OF CONTENTS

1. BACKGROUND

1,2-Benzisothiazol-3(2H)-one (CAS No 2634-33-5, EC No 220-120-9) with the INCI name benzisothiazolinone is currently not listed in Annex VI of the Cosmetics Directive and therefore cannot be used as preservative.

COLIPA has requested the inclusion of benzisothiazolinone in Annex VI in order to allow the use of benzisothiazolinone in cosmetic products.

A first scientific opinion (SCCNFP/0811/04) was adopted 1 July 2004 by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers with the following opinion:

"The SCCNFP is of the opinion that the information submitted is insufficient to assess the safe use of benzisothiazolinone.

Before any further consideration, the following information is required:

* *percutaneous absorption study in accordance with the SCCNFP Notes of Guidance;*
* *reproduction toxicity data."*

The present submission II provides the data requested by this opinion.

2. TERMS OF REFERENCE

1. *Does SCCS consider benzisothiazolinone safe when used as a preservative up to a maximum authorised concentration of 0.01% in cosmetic products, based on the provided data?*

2. *And/or does the SCCS have any scientific concern with regard to the use of benzisothiazolinone in cosmetic products?*

3. OPINION

3.1. Chemical and Physical Specifications

Benzisothiazolinone is listed in the EU Cosmetics Inventory, Section 1 with indicated function "antimicrobial". It is currently not regulated in the annexes of the Cosmetics Directive 76/768/EEC.

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Benzisothiazolinone (INCI)

3.1.1.2. Chemical names

1,2-Benzisothiazol-3(2H)-one (IUPAC)
1,2-Benzisothiazol-3-one
1,2-Benzisothiazolin-3-one
Benzo[a]isothiazol-3-one

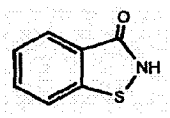
3.1.1.3. Trade names and abbreviations

BIT; Thor BIT; ACTIDE® BIT; Microcare® BIT; Nuosept BIT Technical; Promex BIT COLIPA P96

3.1.1.4. CAS / EC number

CAS: 2634-33-5
EC: 220-120-9

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C_7H_5NOS

3.1.2. Physical form

Off-white to yellowish solid

3.1.3. Molecular weight

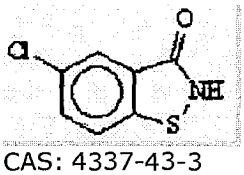
Molecular weight: 151.19 g/mol

3.1.4. Purity, composition and substance codes

Substance code:	/
Batches used:	batch no 2001 014
Purity:	74.02-84.02% w/w (84% corresponds to 15% water content) > 99% w/w on a dry basis
Loss on drying:	/
Water content:	15-29% w/w (for batch no 2001 014 spec., 20% max, found 15%)
Ash content:	/
Sodium chloride:	< 0.1% w/w (for batch no 2001 014 spec., 0.2% max, found 0.02%)
Lead:	/
Mercury:	/
Arsenic:	/
Iron:	/

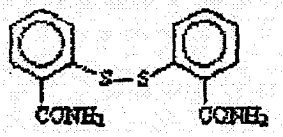
Impurities

- 5-Chloro-1,2-benzisothiazolin-3(2H)-one: 0.15-0.22% w/w
  (for batch no 2001 014, specification 4 ppm max, found 3 ppm)



CAS: 4337-43-3
EC: 224-385-1
emp. Formula: C_7H_4ClNOS

- 2,2-Dichlorobisbenzamide: < 0.1% w/w
  (for batch no 2001 014, specification 0.5% max, found 0.03%)



CAS: 2527-57-3
EC: 219-766-4
emp. Formula: $C_{14}H_{12}N_2O_2S_2$

Residual solvents: /

3.1.5. Impurities / accompanying contaminants

See 3.1.4.

3.1.6. Solubility

Solubility in water: 1.1 g/l (0.11%) at 20 °C
6.0 g/l (0.60%) at 30 °C (Directive 92/69/EEC, A6)

Effect of pH and temperature on solubility in water (OECD Guideline 105)

at 10 °C and pH 4.8: 0.736 g/l
at 20 °C and pH 4.8: 0.938 g/l

at 30 °C and pH 4.8: 1.198 g/l
at 20 °C and pH 6.7: 1.288 g/l
at 20 °C and pH 9.1: 1.651 g/l

3.1.7. Partition coefficient (Log Pow)

Log Pow: 0.4 at 20 °C (OECD Guideline 117)

Effect of pH and temperature on Log Pow (OECD Guideline 117 (HPLC))

Log Pow: 0.99 at 20 °C and pH 5
Log Pow: 0.63 at 10 °C and at pH 7
Log Pow: 0.70 at 20 °C and pH 7
Log Pow: 0.76 at 30 °C and pH 7
Log Pow: – 0.90 at 20 °C and pH 9

Conclusions
With increasing pH from 5 to 9, the Log Pow decreases very strongly. Only a slight increase of Log Pow is observed between 10 °C and 30 °C.

3.1.8. Additional physical and chemical specifications

Melting point:	156.6 °C (Directive 92/69/EEC, A1)
Boiling point:	327.6 °C (Directive 84/449/EEC, A2)
Density:	1.483 g/cm³ at 20 °C (OECD Guideline 109)
Vapour Pressure:	0.0000037 hPa at 25 °C (Directive 92/69/EEC, A4)
pKa:	7.3 at 25 °C
Flash point:	/
Viscosity:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm):	/

3.1.9. Homogeneity and Stability

No data

General Comments to physico-chemical characterisation

No data on stability are provided

3.2. Function and uses

Benzisothiazolinone is listed in the EU Cosmetics Inventory, Section 1 with indicated function "antimicrobial". It is currently not regulated in the annexes of the Cosmetics Directive 76/768/EEC.

The submitted dossier requests a maximum level of 100 ppm as preservative in cosmetic products. This is greater than the level proposed in the earlier submission, which the applicant justifies with the availability of more recent studies, demonstration of a greater margin of safety.

Aside from its use in cosmetic products, there are also other uses:

Under the Biocide review programme for existing substances (Commission Regulation (EC) No 1451/2007), Benzisothiazolinone is examined for use in the following product types:

Private area and public health area disinfectants and other biocidal products (2), In-can preservatives (6), Film preservatives (7), Fibre, leather, rubber and polymerised materials preservatives (9), Masonry preservatives (10), Preservatives for liquid-cooling and processing systems (11), Slimicides (12), Metalworking-fluid preservatives (13), Embalming and taxidermist fluids (22).

Benzisothiazolinone is used as a slimicide in the manufacture of disposable powder-free polyvinyl chloride gloves.

(Add. ref. 41)

Benzisothiazolinone is widely used in industry as a preservative in water-based solutions such as pastes, paints and cutting oils.

(Add. ref. 42, 43)

3.3. Toxicological Evaluation

Throughout the studies quoted (unless otherwise specified) the purity of the test material used was 99.02%, with an active content of 70.02 to 84.02% w/w, water content of 15% to 29% w/w and total impurities of 0.26 to 0.33% w/w.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/08441/04

Guideline:	/
Method:	EPA OPP 81-1
Species/strain:	Rat, Sprague-Dawley derived, albino
Group size:	30 (3 groups of 5 males and 5 females each)
Test item:	Benzisothiazolinone
Test substance:	Nuosept BIT Technical
Batch:	# 170-138 (PSL Code no E50629-1R (powder)
Purity:	1,2-Benzisothiazolin-3-one 82.3%; water 17.7%
Dose:	1000, 2000 and 5000 mg/kg bw/day test substance (823, 1646 and 4115 mg/kg active ingredient)
Vehicle:	Water; the test substance was applied as 40% w/w suspension in water
Route:	oral intubation/gavage
GLP:	in compliance

Results
Based on the findings, the Acute Oral Defined LD50 of Nuosept BIT Technical, Lot #170-138 calculated by Probit Analysis was 1450 milligrams of the test substance per kilogram of bodyweight (when administered as a 40% w/w suspension in distilled water) with 95% Confidence Limits of 2004 mg/kg bw (upper) and 1.049 mg/kg bw (lower). The LD50 for males was 2.100 mg/kg bw with 95% Confidence Limits of 5.029 mg/kg bw (upper) and 877 mg/kg bw (lower). The data does not permit calculation of the LD50 for females by Probit Analysis. Graphically, the LD50 for females was estimated to be 1.050 mg/kg bw.

Ref.: 10

3.3.1.2. Acute dermal toxicity

Taken from SCCNFP/08441/04

Guideline:	/
Method:	EPA OPP 81-2
Species/strain:	rat, Sprague-Dawley derived, albino
Group size:	10 (5 male/5 female)
Test item:	benzisothiazolinone
Test substance:	nuosept BIT Technical
Batch:	# 170-138 (PSL Code no E50629-1R (powder)
Purity:	1,2-Benzisothiazolin-3-one 82.3%; water 17.7%
Dose:	5000 mg/kg bw/day (Limit test) (4115 mg/kg active ingredient)
Vehicle:	water; the test substance was moistened with water for application (1 ml water/1 g test substance)
Route:	topical application (24 h)
Exposure period:	1 x 24 h. observation period 14 d
GLP:	in compliance

Results
An Acute Dermal Toxicity test was conducted with rats to determine the potential for Nuosept Bit Technical, Lot # 170-138 to produce toxicity after topical application. Based on the results of testing, the single dose Acute Dermal Toxicity LD50 of the test substance is greater than 5000 mg/kg bw when applied as received, moistened with distilled water.
5000 mg of the test substance per kilogram of bodyweight was moistened with distilled water and applied to the skin of ten healthy rats (224-232 g) for 24 hours. The animals were observed for signs of gross toxicity and mortality at least once daily for another 14 days. Bodyweights were recorded just prior to application and again on days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.
All animals survived, gained weight and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. Gross necropsy findings at terminal sacrifice were generally unremarkable.

Ref.: 11

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCNFP/08441/04 *(with some modifications)*

Guideline:	/
Method:	EPA OPP 81-5
Species/strain:	New Zealand albino rabbits
Group size:	6, 3 males and 3 females
Test substance:	Nuosept BIT Technical
Batch:	# 170-138
Purity:	1,2-Benzisothiazolin-3-one, 82.3%; Water, 17.7%
Dose:	Slurry of 0.5 g in 0.5 ml water
Exposure:	Semi occlusive
Exposure time:	4 hour(s)
Readings:	1, 24, 48, 72 hours and 7 days
GLP:	in compliance

Date: 1995

Results
The test substance was moistened with water for application (0.5 ml water/0.5 g test substance =41.15% a.i.). One hour after patch removal, well-defined moderate erythema and oedema was noted at all treated sites. This decreased with time. Desquamation occurred at one site and all animals were free of erythema and oedema by day 7.

Conclusion
Nuosept BIT Technical (Benzisothiazolinone) is a skin irritant.

Ref.: 15

Human study

50 healthy human volunteers (20 males and 30 females aged between 19-60 years) were recruited for a randomised double blind open epicutaneous application study to compare the effects of a cream with and without the preservative Microcare® SI. The protocol was approved by an independent ethics committee and the test was performed in compliance with the principles of the Declaration of Helsinki. The test substance was Microcare® SI - an aqueous blend of 2.5% of methylisothiazolinone (MIT) and 2.5% of benzisothiazolinone (BIT).
The test cream contained Microcare® SI at a level of 0.3% w/w of which 0.15% w/w (75 ppm) was 1,2-benzisothiazolinone. Analysis of the test products showed that the formulation contained slightly lower concentrations of isothiazolinones than expected, equivalent to an addition rate of 0.2-0.25%, such as would be used in cosmetic applications.
Subjects with known sensitivity to isothiazolinones were excluded. 47 subjects completed the study as planned, and diary cards and product weights were available. Three subjects withdraw for reasons not related to the study. The test subjects applied twice daily for 4 weeks 1.5 ml of test cream and vehicle to the inner aspects of both forearms in a randomised and blinded fashion. The test sites were assessed after 2 and 4 weeks and the skin reactions scored according to a 5 point ranking scale.

Results
7 subjects reported redness or itching, tingling or stinging sensation upon application of the test cream. The reaction was reported to disappear after the product had been 'absorbed' into the skin. Determining causation demonstrated that all 7 subjects experienced sensations following application of the base cream whereas only 5 experienced sensations following the test cream.

Conclusion
A skin cream preserved with a mixture of MIT/BIT 1:1 at a concentration of 75 ppm of each active was tolerated as well as the vehicle cream under the conditions of the test.

Ref.: 22

3.3.2.2. Mucous membrane irritation

Taken from SCCNFP/08441/04

Guideline:	/
Method:	EPA OPP 81-4
Species/strain:	New Zealand albino rabbits
Group size:	9 (4 males and 5 females)
Test substance:	Nuosept BIT Technical
Batch:	# 170-138
Purity:	1,2-Benzisothiazolin-3-one, 82.3%; Water, 17.7%

Dose:	0.1 g of the undiluted test substance was instilled into the right eye. The treated eyes of 3 rabbits were rinsed 20-30 seconds after instillation; the eyes of the remaining 6 animals were not rinsed.
Exposure time:	48 hours
GLP:	in compliance
Date:	1995

Results
From 1 to 48 hours, all treated eyes exhibited severe to maximal irritation including corneal opacity, iritis and conjunctivitis. Overall the severity of irritation increased with time. Due to the irreversible nature of the irritation the test was terminated after 48 hours.

Conclusion
The test substance was severely irritating to the rabbit eye.

Ref.: 17

An assessment of the eye irritancy potential of 1,2-benzisothiazolin-3-one using the Bovine Corneal Opacity and Permeability assay *in vitro*

Guideline:	/
Method:	INVITTOX Protocol 124
Species:	Bovine
Number corneas:	45
Test substance:	1,2-Benzisothiazolin-3-one
Batch:	LHW 1355
Purity:	>99%
Dose:	75, 750 and 7500 ppm aqueous solution of BIT prepared as its sodium salt
Exposure time:	10 minutes
GLP:	in compliance
Date:	2003

Bovine eyes mounted on holders and incubated with Minimal Essential Medium (MEM) were exposed to test article or positive or negative control. After 10 minutes exposure the corneas were rinsed and again incubated with media.
Corneal opacity was measured with an opacimeter and corneal permeability was determined using sodium fluorescein and measured spectrophotometrically. The corneal opacity and permeability were combined to give an in-vitro score

Results
The mean in vitro score for BIT at 7500 ppm was 3.012, at 750 ppm 0.666 and at 75 ppm -0.207, compared with 0.490, 0.416 and 0.449 for saline (negative control) and 50.73, 50.23 and 50.25 for ethanol (positive control), respectively.

Conclusion
Benzisothiazolinone was considered to be non-irritant to the eye at all tested concentrations in the BCOP assay under the conditions of the test.

Ref.: 18

Comment
The method used was not a validated *in vitro* method in 2003. Meanwhile, it is an OECD test guideline (number) which can detect strong irritants.

An assessment of the cytotoxicity of 1,2 benzisothiazolin-3-one by *in vitro* Neutral Red Uptake Assay using BalbC 3T3 & SIRC mammalian cell lines

Guideline:	/
Species:	BALB/c 3T3 Mouse fibroblast cell line SIRC Rabbit corneal cell line
Test substance:	1,2-Benzisothiazolin-3-one
Batch:	LHW 1355
Purity:	> 99%
Dose:	0-100 ppm range finding; 0-10 ppm testing
Exposure time:	24 hours
GLP:	in compliance
Date:	2002

Results
The effects of the test substance on cell viability of the two different cell lines was measured by the neutral red uptake. A best-fit dose-response curve for each set of experiments was calculated from the data using non-linear regression and the respective EC50 value was calculated. The EC50 for 3T3 cells was 3.1414 ppm and that for SIRC cells was 3.6666.
These results may be compared with results previously obtained for other preservative preparations, as illustrated in the following table demonstrating that BIT is less cytotoxic than CIT/MIT, yet more cytotoxic than other commonly used preservatives.

Cytotoxicity values for cosmetic preservatives

Cosmetic Preservative	EC50 3T3	EC50 SIRC
Chloromethylisothiazolinone/methylisothiazolinone (3:1)	1.19	1.89
Methylisothiazolinone	5.76	5.98
Methyldibromoglutaronitrile 20% (in phenoxyethanol)	28.4	29.36
Methyl/ethyl/propyl/butyl/isobutyl parabens in phenoxyethanol	439.5	489.0

Ref.: 19

Comment
The SCCS considers the above cytotoxicity data of little value in its overall evaluation of the safety of benzisothiazolinone.

3.3.3. Skin sensitisation

Taken from SCCNFP/08441/04

Guinea Pig Maximization Test (Magnusson and Kligman)

Guideline:	OECD 406
Species/ strain:	Albino Dunkin Hartley guinea pigs
Size:	38 (20 test, 10 control, 8 range-finding)
Test substance:	1,2-benzisothiazolin-3-one 79.8%, water 19.2%
Batch:	386-3
Purity:	79.8% 1,2-benzisothiazolin-3-one (BIT), water, 19.2% Diamide content 0.28%, PCP < 1 ppm.
Dosage:	1st induction 0.1% w/v intracutaneous 2nd induction 20% w/v occlusive epicutaneous 3rd challenge 10% w/v occlusive epicutaneous
Vehicle:	corn seed oil (1st and 2nd concentration), FCA/water (1st concentration), Ethanol (3rd concentration)
GLP:	in compliance
Date:	1996

Results
Results from 2 animals in range-finding studies indicated that 0.1% w/v in cottonseed oil should be used for intradermal induction.
In topical range-finding studies in 4 animals, it was indicated that 20% in cottonseed oil was minimally irritant and was suitable for topical induction. In further topical range-finding studies in 2 animals it was found that 10% in ethanol was suitable for challenge.
Following challenge, 9 out of 20 animals in the test group reacted positively to 10% w/v test article in ethanol at 24 or 48-hour examinations, giving a response incidence of 45%.

Conclusion
BIT is a moderate contact sensitizer.

Ref.: 20

Buehler Method

Guideline:	/
Method:	EPA OPP 81-6
Species/strain:	Hartley albino guinea pigs
Size:	8 for range finding, 30 for test protocol
Test substance:	Nuosept Bit technical (82.3% 1,2-Benzisothiazolin-3-one, water 17.7%)
Batch:	#170-138
Purity:	1,2-Benzisothiazolin-3-one (BIT) 82.3% a.i.
Dosage:	Induction: Weekly application of 0.3 g of test substance 95% w/w in corn seed oil for 3 consecutive weeks. Challenge: 14 days after last induction with same dose as induction to naive site
Vehicle:	Corn seed oil
GLP:	in compliance
Date:	1995

Results
It was found that a 6 hour exposure under 25 mm Hilltop chambers to 95% w/w (78.19% active) BIT powder in corn oil was suitable for the test group. 0.04% DNCB (dichloronitro-benzene) in acetone was used as the positive control.
No reaction was seen at any test or naive control site following challenge. 7/10 positive control animals exhibited signs of reaction to challenge at 24 hours. This reaction persisted in 5 animals at 48 hours.

Conclusion
Nuosept BIT Technical was not a sensitizer in this test.

Ref.: 21

Human study

15 healthy human volunteer patients (2 males and 13 females aged between 25-66 years) were recruited for a randomised double blind open epicutaneous application study to compare the effects of a cream with and without the preservative Microcare® SI. The volunteer subjects were previously diagnosed as being sensitized to chloromethyliso-thiazolinone.
The test cream Doublebase™ contained Microcare® SI at a level of 0.3% w/w (150ppm) of which 0.15% w/w (75ppm) was 1,2-benzisothiazolinone.
The test subjects were instructed to apply twice daily for 4 weeks 1-1.5 ml of test cream and vehicle to the inner aspects of both forearms in a randomised and blinded fashion. They were asked to complete a diary to record usage of the products, which were weighed at the end of study.

Results
10 subjects completed the study as planned, and diary cards and product weights were available. Three subjects were withdrawn from the study due to adverse reactions, 2 subjects after less than 7 days and 1 after 21 days. They all noticed flare of eczema on their forearms. Two subjects were lost to follow up. After four weeks application, the frequency of each assessment grade is summarised as follows.

Assessment grading Product Code	Product Code	
	1	2
Withdrawn	3	3
No Assessment	2	2
No Visible Redness	9	10
Slight Redness	0	0
Distinct Redness	1	0
Total	15	15

There were three cases of flare of eczema related to the study preparations.
Two were associated with the application of product 1, and one with product 2.

Ref.: 23

Comments
The study included a low number of test subjects; there was no detailed description of their existing chloromethylisothiazolinone (CMI) allergy, lack of assessment of skin reactions under the 4 week study period, and a significant variation in product usage, from around 35 grams to 110 grams per test period. Limited conclusions can be drawn. The test products seem to elicit dermatitis in some test subjects.

Local Lymph Node Assay and Human Repeat Insult Patch Test

The relative sensitising potencies and potential for cross sensitisation/reaction of chloromethylisothiazolinone (CMI or CIT/MIT), methyl trimethylene isothiazolinone (MTI) and benzisothiazolinone (BIT) were considered from newly generated and historical data. However, although CMI was specifically mentioned, it was not stated whether the mixture of chloromethylisothiazolinone with methylisothiazolinone was actually used. Original experimental data were not provided in this review.

Using the LLNA, the EC3 for Benzisothiazolinone was established at 10.4%, that for MTI at 2% and CIT/MIT at 0.01%.

Using the HRIPT resulted in no reactions to BIT at 360 ppm and 9% of volunteers reacting at 725 ppm. There were also no reactions to CMIT/MIT at 10 ppm and 4.4% reacted at 20 ppm. For MTI there were no reactions at 100 ppm but 16% reacted at 300 ppm.

Data are summarized:

Preservative	Test concentration (ppm)	Proportion of human panel sensitised (%)	LLNA EC3
BIT	725	5/58 (9%)	10.4%
BIT	360	0/54 (0%)	
MTI	300	3/19 (16%)	2.0%
MTI	100	0/211(0%)	
CIT/MIT	20	2/45 (4.4%)	0.007-0.01%
CIT/MIT	10	0/175(0%)	

Ref.: 24

Comment
The HRIPT is not considered ethical.

Local Lymph Node Assay
An EC3 of 2.3% for 1,2-benzisothiazolin-3-one (CAS 2634-33-5) and 1.9% for 2-methyl-2H-isothiazol-3-one (CAS 2682-20-4) is tabulated in a review but no experimental details are provided.

(Add. ref.: 49)

Overall Comment Sensitisation

Benzisothiazolinone is a contact allergen with the guinea pig maximization (Magnusson Kligman) test indicating BIT as a moderate sensitiser, as does the LLNA with an EC3 of 2.3% for BIT.

Since the human in-use study provided by the applicant included a low number of subjects, only limited conclusions could be drawn. Therefore, the SCCS conducted a literature search on the sensitising potential of benzisothiazolinone in humans. From the dermatological literature case reports describe allergic contact dermatitis to benzisothiazolinone. It is a well-documented contact allergen. However, its potency is lower than other marketed cosmetic preservatives, and the irritancy profile makes it a difficult contact allergen to test with.

(Add. ref.: 37, 38, 39, 40)

Benzisothiazolinone is widely used in industry as a preservative in water-based solutions such as pastes, paints and cutting oils. Occupational dermatitis has been reported mainly due to cutting fluids, paint manufacture, pottery mould-makers, acrylic emulsions manufacture, printers, paper makers etc. which contain benzisothiazolinone.

(Add. ref. 42, 44, 45, 46, 47, 48)

Moreover, benzisothiazolinone is used as a slimicide in the manufacture of disposable powder-free polyvinyl chloride (PVC) gloves. Of 31 glove brands studied 9 (30%) contained 3–26 ppm benzisothiazolinone. Individuals wearing such gloves developed allergic contact dermatitis. A concentration of 20 ppm benzisothiazolinone in a glove seems to be enough for sensitization.

(Add. ref. 41)

3.3.4. Dermal / percutaneous absorption

New study

Guideline:	OECD 428
Species/strain:	human skin (5 abdominal, 1 breast); 400 µm dermatomed skin
Group size:	10 membranes
Method	flow through diffusion cell
Membrane integrity	Tritiated water
Test substance:	benzisothiazolinone
Batch:	12409EE
Purity:	97%
Radiochemical	^{14}C benzisothiazolinone; 99.9%
Vehicle:	water
Test item:	0.01% w/v benzisothiazolinone aqueous
Dose volume:	20 µl/cm^2
Receptor	Tissue culture medium containing 6% PEG, 0.01% sodium azide, 1% glucose and streptomycin
Solubility	(in water) 1 mg/ml
Method of Analysis:	liquid scintillation
GLP:	in compliance
Study period:	2008

The absorption of radiolabelled 0.01% w/v benzisothiazolinone in aqueous solution was determined from the use of 10 chambers with human dermatomed skin.

: 1 **Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [^{14}C]-Benzisothiazolinone in Water (0.01%, w/v) to Human Split-Thickness Skin**

	Cell Number and Donor Number											
	Cell 1 0221	Cell 2 0221	Cell 3 0223	Cell 4 0223	Cell 5 0223	Cell 6 0225	Cell 7 0225	Cell 8 0225	Cell 13 0233	Cell 14 0233	Mean	SD
Skin Wash	35.87	11.66	8.11	12.00	8.59	17.56	9.41	20.10	16.14	18.59	15.80	8.27
Cell Wash	0.52	0.19	0.26	0.21	0.22	0.42	1.28	0.59	0.56	0.58	0.48	0.32
Tissue Swab	24.53	31.27	18.50	19.66	22.57	22.69	24.20	22.21	29.02	42.96	25.76	7.17
Pipette Tip	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Dislodgeable Dose	60.92	43.14	26.87	31.87	31.38	40.68	34.90	42.91	45.73	62.14	42.05	11.91
Stratum Corneum 1-5	0.83	1.44	0.30	1.89	2.25	0.83	1.20	0.94	4.53	1.03	1.52	1.20
Stratum Corneum 6-10	0.53	0.82	0.46	2.48	1.36	1.12	1.45	1.86	4.00	0.64	1.47	1.09
Stratum Corneum 11-15	0.25	0.67	0.59	1.58	1.45	1.33	0.76	1.41	1.71	0.84	1.06	0.49
Stratum Corneum 16-20	0.20	0.79	0.48	0.99	1.10	1.21	0.75	0.62	2.19	0.57	0.89	0.55
Stratum Corneum	1.81	3.71	1.82	6.94	6.16	4.48	4.15	4.82	12.43	3.08	4.94	3.11
Unexposed Skin	0.01	*0.00	0.02	0.01	*0.00	0.00	0.15	0.03	*0.00	*0.00	*0.02	*0.05
Total Unabsorbed	62.75	46.85	28.71	38.82	37.54	45.17	39.20	47.77	58.16	65.22	47.02	11.83
Epidermis	12.14	25.12	32.41	24.50	23.20	12.16	16.18	12.03	23.52	16.16	19.74	6.98
Dermis	0.66	6.94	7.25	4.17	2.88	4.11	8.47	3.27	6.12	0.70	4.46	2.70
Receptor Fluid	22.09	17.78	28.53	29.85	32.46	34.91	32.92	33.99	7.56	14.10	25.42	9.52
Receptor Rinse	0.06	0.11	0.32	0.28	0.34	0.18	0.28	0.26	0.16	0.14	0.21	0.09
Total Absorbed	22.15	17.88	28.85	30.13	32.79	35.09	33.20	34.25	7.72	14.24	25.63	9.58
Dermal Delivery	34.95	49.94	68.52	58.80	58.87	51.36	57.85	49.55	37.36	31.10	49.83	12.05
Mass Balance	97.69	96.80	97.23	97.62	96.41	96.53	97.05	97.32	95.52	96.31	96.85	0.67

Distribution of [^{14}C]-Benzisothiazolinone (µg equiv./cm^2) at 24 h Post Application Following Topical Application of [^{14}C]-Benzisothiazolinone in Water (0.01%, w/v) to Human Split-Thickness Skin

	Cell Number and Donor Number											
	Cell 1 0221	Cell 2 0221	Cell 3 0223	Cell 4 0223	Cell 5 0223	Cell 6 0225	Cell 7 0225	Cell 8 0225	Cell 13 0233	Cell 14 0233	Mean	SD
Skin Wash	0.75	0.24	0.17	0.25	0.18	0.37	0.20	0.42	0.34	0.39	0.33	0.17
Cell Wash	0.01	0.00	0.01	0.00	0.00	0.01	0.03	0.01	0.01	0.01	0.01	0.01
Tissue Swab	0.51	0.65	0.39	0.41	0.47	0.47	0.51	0.46	0.61	0.90	0.54	0.15
Pipette Tip	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dislodgeable Dose	1.27	0.90	0.56	0.67	0.66	0.85	0.73	0.90	0.96	1.30	0.88	0.25
Stratum Corneum 1-5	0.02	0.03	0.01	0.04	0.05	0.02	0.03	0.02	0.09	0.02	0.03	0.02
Stratum Corneum 6-10	0.01	0.02	0.01	0.05	0.03	0.02	0.03	0.04	0.08	0.01	0.03	0.02
Stratum Corneum 11-15	0.01	0.01	0.01	0.03	0.03	0.03	0.02	0.03	0.04	0.02	0.02	0.01
Stratum Corneum 16-20	0.00	0.02	0.01	0.02	0.02	0.03	0.02	0.01	0.05	0.01	0.02	0.01
Stratum Corneum	0.04	0.08	0.04	0.15	0.13	0.09	0.09	0.10	0.26	0.06	0.10	0.06
Unexposed Skin	0.00	*0.00	0.00	0.00	*0.00	0.00	0.00	0.00	*0.00	*0.00	*0.00	*0.00
Total Unabsorbed	1.31	0.98	0.60	0.81	0.78	0.94	0.82	1.00	1.21	1.36	0.98	0.25
Epidermis	0.25	0.52	0.68	0.51	0.48	0.25	0.34	0.25	0.49	0.34	0.41	0.15
Dermis	0.01	0.14	0.15	0.09	0.06	0.09	0.18	0.07	0.13	0.01	0.09	0.06
Receptor Fluid	*0.46	*0.37	*0.60	*0.62	*0.68	*0.73	*0.69	*0.71	*0.16	*0.29	*0.53	*0.20
Receptor Rinse	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00
Total Absorbed	0.46	0.37	0.60	0.63	0.68	0.73	0.69	0.72	0.16	0.30	0.54	0.20
Dermal Delivery	0.73	1.04	1.43	1.23	1.23	1.07	1.21	1.03	0.78	0.65	1.04	0.25
Mass Balance	2.04	2.02	2.03	2.04	2.01	2.02	2.03	2.03	2.00	2.01	2.02	0.01

Test Preparation Vehicle	Water	
Target Test Item Concentration in Test Preparation (%, w/w)	0.01	
Actual Test Item Concentration in Test Preparation (%, w/w)	0.0104	
Target Application Rate of Test Preparation (mg/cm^2)	20	
Actual Application Rate of Test Preparation (mg/cm^2)	20.06	
Target Application Rate of Test Item (µg equiv./cm^2)	2	
Actual Application Rate of Test Item (µg equiv./cm^2)	2.09	
Distribution	Mean	SD
Total Dislodgeable Dose (% Applied Dose)	42.05	11.91
Unabsorbed Dose (% Applied Dose)	47.02	11.83
Absorbed Dose (% Applied Dose)	25.63	9.58
Dermal Delivery (% Applied Dose)	49.83	12.05
Mass Balance (% Applied Dose)	96.85	0.67
Dislodgeable Dose (µg equiv./cm^2)	0.88	0.25
Unabsorbed Dose (µg equiv./cm^2)	0.98	0.25
Absorbed Dose (µg equiv./cm^2)	0.54	0.20
Dermal Delivery (µg equiv./cm^2)	1.04	0.25
Mass Balance (µg equiv./cm^2)	2.02	0.01

Total unabsorbed dose = skin wash + tissue swab + pipette tips + stratum corneum + unexposed skin + cell wash
Absorbed dose = cumulative receptor fluid + receptor rinse
Dermal delivery = exposed skin (epidermis + dermis) + absorbed dose
Mass balance = unabsorbed dose + dermal delivery

Conclusion
Following topical application of [^{14}C]-Benzisothiazolinone in water (0.01%, w/v) to human skin, the absorbed dose of [^{14}C]-Benzisothiazolinone was 25.63% (0.54 µg equiv./cm^2). The dermal delivery of [^{14}C]-Benzisothiazolinone was 49.83% (1.04 µg equiv./cm^2). The total dislodgeable dose was 42.05% of the applied dose.

Ref.: 36

Comment
This was a properly performed study. Therefore, the mean + 1SD may be used for calculating the MOS. This is 61.9% of the applied dose (1.29 µg/cm^2) when 0.01% benzisothiazolinone aqueous was applied. The dermal absorption studies had not been performed with representative cosmetic formulations.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Taken from SCCNFP/08441/04 *(with modifications)*

Guideline:	OECD 407
Species/strain:	Rat, Wistar Hsd Cpb:WU
Group size:	12 (6 male/6 female)
Test item:	Benzisothiazolinone, Code 072/1-PBP
Test substance:	Promex BIT (paste)
Purity:	1,2-Benzisothiazolin-3-one, 84.29%; water, 15%, purity of active ingredient on dry weight basis, 99.02%
Batch:	2001 014//sample no. KP 070601//cb 181100
Dose levels:	0, 15, 45 and 135 mg/kg bw/day (12.63, 37.89 and 113.67 mg/kg a.i.), suspended in 0.5% CMC
Route:	daily oral intubation/gavage
Exposure period:	28 days
GLP:	in compliance
Date:	2001

Results

Oral administration of 1,2-Benzisothiazolin-3-one, by gavage in Wistar rats at the dose of 15 mg/kg bw/day (12.63 mg a.i./kg bw/day) had no adverse effect on general health, neurological effects, growth, food consumption, haematological and clinical chemistry parameters, organ weights and its ratios, gross and histopathological changes.
Treatment related signs of slight salivation were observed in all the males in the main group at 135 mg/kg bw/day and its recovery group from treatment day 17 and in two females from the test group and two in the recovery group from treatment day 20 till the end of the treatment period. During the recovery period, treatment related signs of salivation were not observed in the 135 mg/kg bw/day group indicating the reversibility of the effect.
Body weight was unaffected in the 15 and 45 mg/kg bw/day groups. At 135 mg/kg bw/day there was a significant decrease in body weight and cumulative net weight gain in the male group throughout the treatment period with the exception of the first week where it was not statistically significant.
Weekly body weights were significantly lower throughout the treatment and recovery period in the high dose (135 mg/kg bw/day) for males and on weeks 4, 5, and 6 for females.
Cumulative net weight gains were also significantly lower throughout the treatment and recovery period in males and females with the exception of the first week where it was not statistically significant.
Increased incidences of histopathological lesions in the non-glandular stomach (hyperkeratosis, epithelial hyperplasia, ulceration) were observed in mid- (45 mg/kg bw/day) and high-dose (135 mg/kg bw/day) males and females. The severity of the lesions was reduced in the high-dose recovery group.

The NOAEL in this study was 15 mg/kg bw/day (12.63 mg a.i./kg bw/day).

Ref.: 12

Comment
The SCCS noted that the NOAEL of 15 mg/kg bw/day (12.63 mg a.i./kg bw/day) was based on the histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance.

3.3.5.2. Sub-chronic (90 days) toxicity (oral, dermal)

Taken from SCCNFP/08441/04

Guideline:	OECD 408
Species/strain:	Rat, Wistar
Group size:	Total 20; 10 male/10 female
Test item:	Benzisothiazolinone (Promex-BIT)
Batch:	G00Z-0600-1907-13//072/1.PBP//2001 014//KP 070601
Purity:	1,2-Benzisothiazolin-3-one, 84.29%; water 15%; purity of active ingredient on dry weight base 99.1%
Dose levels:	10, 30 and 75 mg/kg bw/day (8.42, 25.26 and 63.15 mg/kg bw/day a.i.)
Vehicle:	0.5% carboxymethylcellulose (CMC)
Route:	daily oral intubation/gavage
Exposure period:	90 days
GLP:	in compliance
Study period:	2001-2002

Results
The oral administration of 1,2-Benzisothiazolin-3-one by gavage in Wistar rats at the dose of 10 mg/kg bw/day (8.42 mg a.i./kg bw/day) had no adverse effect on general health, neurological effects, growth, food consumption, haematological and clinical chemistry parameters, sperm evaluation, organ weights and its ratios and gross and histopathological changes.

At 30 mg/kg bw/day (25.26 mg a.i./kg bw/day) there were no treatment related clinical signs or neurological effects and no adverse effects on growth, haematological and clinical chemistry parameters, sperm evaluation, organ weights and its ratios. Food consumption was lower in females in weeks 2 and 4. There were some changes primarily in the non-glandular stomach region both macroscopically and histologically which were considered treatment related and were reversible. These effects may have been due to the irritant nature to the test substance. Increased incidences of histopathological lesions in the non-glandular stomach (hyperkeratosis, epithelial hyperplasia, ulceration) were observed in males and females.
At 75 mg/kg bw/day (63.15 mg a.i./kg bw/day) there were no treatment related neurological effects and no adverse effects on haematological and clinical chemistry parameters, sperm evaluation, organ weights and its ratios. Treatment related signs of slight salivation were observed in four males and three females in the main group and in three males and five females in the recovery group during the treatment period. During the recovery period, treatment related signs of salivation were not observed indicating the reversibility of the effect. Weekly body weights and cumulative net weight gains were significantly lower in males throughout the treatment and recovery period except for the body weight at week 3 which were lower but not statistically significant. The body weight gains were significantly higher in males and in females (weeks 15, 16 and 17) during the recovery period. There was a significant reduction in food consumption in males (weeks 1, 2, 7, 8, 12 and 13) and in females (weeks 1, 2 and 4), which returned to control levels in the recovery period.
Increased incidences of histopathological lesions in the non-glandular stomach (hyperkeratosis, epithelial hyperplasia, ulceration, keratin cysts) were observed in males and females. The severity of the lesions was reduced in the recovery group.

The NOAEL in this study was 10 mg/kg bw/day (8.42 mg a.i./kg bw/day).

Ref.: 13

Comment
The SCCS noted that the NOAEL of 10 mg/kg bw/day (8.42 mg a.i./kg bw/day) was based on the histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance.
Therefore, the NOAEL is 25.26 mg a.i./kg bw/day based on systemic effects.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/08441/04

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA1537, TA100, TA1535 *Escherichia coli* WP2uvrA pkM 101
Test substance:	Promex BIT 1,2–Benzisothiazolin-3-one
Batch:	2001 014
Lot number:	KP 070601
Purity:	99.02%
Concentrations:	0, 20, 35, 60, 100 and 175 µg/plate (1st experiment) 0, 30, 50, 75, 120 and 180 µg/plate (2nd experiment)

Replicate:	3 plates/concentration
Positive controls:	according to guideline
Metabolic activation:	Aroclor induced rat liver homogenate
GLP:	in compliance
Date:	2001

Results
Toxicity: in a preliminary study with a series of concentrations up to 5000 µg/plate, there was a decrease in the mean number of revertants from the concentrations up to 160 µg/plate.
Mutagenicity: only the lowest doses could be evaluated in comparison with the untreated plates (10-20 µg/plate).
The study cannot be used for the evaluation due to the high toxicity of the test item towards the bacterial cells.

Ref.: 25

In vitro Mammalian Cell Gene Mutation Test

Guideline:	OECD 476 (1997)
Species/strain:	CHO-K1 (Chinese hamster ovary cells) HPRT locus
Test substance:	Promex BIT; 1,2-Benzisothiazolin-3-one
Batch:	2001 014
Lot number:	KP 070601
Purity:	99.02%
Concentrations:	0, 0.65, 1.30, 2.60, 5.20 µg/ml
Treatment time:	5 hours, with and without metabolic activation
Replicate:	2 experiments in the same conditions.
Positive controls:	B(a)P; EMS
Metabolic activation:	Aroclor 1254 induced rat liver homogenate.
GLP:	in compliance
Date:	2002

Results
Toxicity: in the presence of metabolic activation a toxic effect produced by the test item between 4 and 6 µg/ml was observed; in the absence of metabolic activation a toxic effect produced by the test item was observed between 2 and 4 µg/ml. The toxic doses reduced the survival to less than 50% of the untreated cells.
Mutagenicity: there was no increase of mutants in the treatment with the test substance, in the presence and in the absence of metabolic activation after 5 hours of treatment.
In the absence of metabolic activation, a treatment of 20 hours was not performed as suggested by the guideline.
The study indicates that the test item is not mutagenic in the condition of the test.

Ref. 26

In vitro Mammalian Chromosome Aberration test

Guideline:	OECD 473 (1997)
Species/strain:	CHO-K1 cell line (Chinese hamster ovary cells)
Test substance:	Promex BIT; 1,2–Benzisothiazolin-3-one
Batch:	2001 014
Lot number:	KP 070601
Purity:	99.02%
Concentrations:	0, 1.6, 3.2, 6.4 µg/ml in the presence of S9-mix 0, 1.25, 2.50, 5.0 µg/ml in the absence of S9-mix
Replicate:	2 experiments (200 metaphases analysed)

Treatment time:	1st experiment: 3 hours 2nd experiment: 3 hours, in the presence of S9-mix; 19 hours, in the absence of S9-mix
Positive controls:	CPA (55 µg/ml); EMS (600 µg/ml)
Metabolic activation:	Aroclor 1254 induced rat live homogenate
GLP:	in compliance
Date:	2001

Results
Toxicity: 2 preliminary experiments showed that the test item was toxic at concentrations between 75 and 5000 µg/ml and between 14 and 58.94 µg/ml.
Clastogenicity: the test item induced chromosome aberrations at the maximum tested dose in the presence of a metabolic activation, and at all concentrations, in the absence of a metabolic activation system.
The test item is clastogenic in CHO mammalian cells.

Ref.: 27

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Taken from SCCNFP/08441/04

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	Swiss albino mice-HsdOla: MF1 strain
Test substance:	Promex BIT; 1,2–Benzisothiazolin-3-one
Batch:	2001 014
Lot number:	KP 070601
Purity:	99.02%
Doses:	63.15; 126.3; 210.5 mg/kg a.i.
Treatment:	oral (gavage) twice, at 24 hours of interval. The animals were sacrificed 24 hours after the second treatment.
Positive control:	CPA, 40 mg/kg bw, oral treatment
GLP:	in compliance
Date:	2001

Results
Toxicity: in a preliminary test, a dose of 250 mg/kg bw was found not toxic (no clinical signs), whereas 450 and 900 mg/kg bw were toxic.
Clastogenicity: in all treated mice, there was a reduction of the ratio PCE/NCE, thus indicating that the test item has reached the target cells.
The positive control, CPA, induced a number of MN significantly higher than the untreated animals. The test item did not induce a number of MN higher than the untreated animals.
The test item is not clastogenic in mice, treated in vivo.

Ref.: 28

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*

Guideline:	OECD 486 (1997)
Species/strain:	Wistar Hanlbm: WIST (SPF) rats
Test substance:	Promex BIT
Batch:	2001014
Purity:	99.02%
Doses:	0 (corn oil), 375, 750 mg a.i./kg bw
Treatment times:	2 hours, 16 hours, orally, once

Positive controls:	N,N'-dimethylhydrazine dihidrochloride (DMH): 40 mg/kg bw; 2 hours 2-acetylaminofluorene (2-AAF): 100 mg/kg bw; 16 hours.
GLP:	in compliance
Date:	2002

Results
Toxicity: in preliminary experiments, doses of 1200 and 1000 mg a.i./kg bw were found toxic to the animals.
DNA repair: autoradiography was done on at least three cultures of hepatocytes per animals.
There was no indication of induction of UDS by the test item. The two positive controls induced a significant increase of UDS.
The test item does not induce UDS in rat hepatocytes in *in vivo* treatment.

Ref.: 29

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity study

Guideline:	EPA Health Effects Test Guidelines OPPTS 870.3800, of aug 1998
Species/strain:	Crl:W1 (Glx/BRL/Han)BR
Group size:	24 males and 24 females (P generation)
Test substance:	Proxel Press Paste (1,2-Benzisothiazolin-3-one)
Batch:	No. 103 and No. 344
Purity:	Purity of active ingredient: 92.7% +/- 1.1%
Dose levels:	P generation: 250, 500 and 1000 ppm F1 generation: 250, 500 1000 ppm
Route:	oral
Administration:	dietary
Control:	control diet only
GLP statement:	Yes
Study period:	2000-2001

Study design
Groups of 24 male and 24 female rats were given Proxel Press Paste by admixture with the diet, at dose levels of 250, 500 and 1000 ppm. A similar group received the control diet only. The animals received the test diet for 10 weeks before being paired for up to 2 weeks. Dosing continued during this pairing period, and throughout the resulting pregnancies. The P generation females were allowed to litter and rear their offspring (F1a) until weaning.
Administration of the test article continued throughout the weaning of the F1 offspring up until necropsy.
24 animals of each sex were randomly selected from each group to form the filial (F1) generation. Direct treatment of the F1 generation continued during their maturation period (10 weeks), the mating period (up to two weeks) and throughout the resulting pregnancies and weaning of the F2 offspring until necropsy. All F1 females were allowed to litter and rear their F2a offspring to weaning.

Result
Analysis of samples from the diets prepared for administration in weeks 1, 17 and 19 of the study showed that the achieved concentrations were within the target range (all values within 89 to 110% of nominal). Analysis of samples from the diets prepared for

administration in weeks 34, 36, 37 and 38 of the study showed that the achieved concentrations were below the target range.

The group mean achieved intakes of PROXEL Press Paste were:

Generation	Dose level (ppm)	Intake (mg/kg/day) Males	Females
P	250	18.5	27.0
	500	37.2	54.2
	1000	75.1	112.0
F_1	250	24.0	28.2
	500	48.0	56.6
	1000	97.8	114.8
Combined	250	21.3	27.6
	500	42.6	55.4
	1000	86.5	113.4

P Generation: Clinical observations, body weights and food intakes were unaffected by treatment. Mating data, duration of gestation, number of implantations, numbers of pups born and pup survival were similar in all groups.
Mean pup weight of the high dose pups was slightly lower than the control over the first week *post-partum*. But, over the whole lactation period, mean pup weight was similar in all groups.
There were no adverse effects of treatment on seminology data.
In the high dose group, mean liver weight of the males was slightly higher, and mean testes weight slightly lower than control.
Minor limiting ridge hyperplasia in the stomach was noted in some intermediate and many high dose animals. Squamous cell hyperplasia and forestomach gastritis was also seen in a few animals.

F1 Generation: Males in the high dose group gained slightly less weight than the controls during the study and the high dose females gained slightly less weight during the pre-pairing period only. Clinical observations and food intakes were unaffected by treatment.
Physical development of the F1 generation, mating data, duration of gestation and F2a pup sex ratio were unaffected by treatment. Pup survival to day 4 *post-partum* and mean pup weight gain were slightly lower in the high dose group compared to controls. Seminology investigations, organ weights and ovarian follicle counts were unaffected by treatment.
In the intermediate and high dose groups, limiting ridge hyperplasia in the stomach was noted. This was most prominent in the high dose females where there was also squamous cell hyperplasia, fore stomach gastritis, hyperkeratosis and erosion/ulcer.

Conclusion
Dietary administration of 1000 ppm PROXEL Press Paste to rats for two generations produced slight adult toxicity, in the F1 generation in terms of lower body weight gain, and in both generations limiting ridge hyperplasia of the stomach together with incidences of squamous cell hyperplasia, forestomach gastritis, hyperkeratosis and erosion/ulcer. At this concentration, the growth of the offspring was slightly impaired and in the F2a offspring, there was a slight reduction in pup survival.
At 500 ppm, there were incidences of limiting ridge hyperplasia in the stomach only.
There were no adverse effects of treatment at 250 ppm, equivalent to an approximate overall mean intake of 24 mg/kg bw/day.
Note: The dose levels refer to the concentration of the active ingredient (BIT) and a correction factor of 1.074 was made for the stated purity of the PROXEL.

Ref.: 14

Comments
No significant effects were reported for the reproductive parameters at any dose level and only slight effects were noted in the offspring at the highest dose level (slightly lower pup survival to day 4 *post-partum* and slightly lower mean pup weight gain).
Based on this study, a NOAEL of approximately 50 mg a.i./kg bw/day is used as a conservative estimate for the MOS calculation. This NOAEL is lower than the lowest LOAEL for systemic effects (63 mg a.i./kg bw/day) in the 90-day study.

3.3.8.2. Teratogenicity

No evidence for teratogenicity in the two-generation oral reprotoxicity study

Ref.: 14

3.3.9. Toxicokinetics

No data provided.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Guideline:	OECD Test guideline 432
Test:	Neutral Red uptake phototoxicity test with Balb/c 3T3 cells
Test substance:	1,2-Benzisothiazolin-3-one
Batch:	LMS 414, CH-0405-ST-7
Purity:	> 99%
UV-A Irradiation:	SOL-500 lamp fitted with a H1-filter mounted at a distance of 60 cm to achieve an UV-irradiance of 5 J/cm^2
Concentrations:	0, eight concentrations between 0.2 to 50 µg/mL in the presence and absence of UV-A exposure
Replicate:	3 main experiments
Positive control:	Chlorpromazine
GLP:	in compliance
Date:	2004

Results
The 3T3 NRU phototoxicity assay was performed on four occasions for BIT, once as a range finding experiment and three times as main experiment. The PIF values calculated from the results for BIT range between 1.197 and 2.166 with a mean value of 1.54. The positive control chlorpromazine yielded PIF values of about 100.

Conclusion
1,2 Benzisothiazol-3(2H)-one is predicted to be non-phototoxic by the 3T3 NRU PT assay OECD Test Guideline 432

Ref.: 16

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

See section 3.3.3. Sensitisation

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Benzisothiazolinone

Daily exposure to all cosmetic products (excl. sunscreens)	**=**	**17.4 g/d**
Concentration Benzisothiazolinone (BIT)	**=**	**0.01%**
Daily exposure BIT	**=**	**1.74 mg**
Dermal absorption	**=**	**61.9%**
Typical body weight of human	**=**	**60 kg**
Systemic exposure dose	**=**	**0.018 mg/kg bw/d**
No Observed Adverse Effect Level (2-generation-study, oral, rat)	**=**	**50 mg/kg bw/d**
NOAEL corrected for 50% oral bioavailability	**=**	**25 mg/kg bw/d**

Margin of Safety	**NOAEL/SED**	**=**	**1392**

3.3.14. Discussion

Physico-chemical properties
Benzisothiazolinone is an off-white to yellowish solid that is soluble in water (1.1 g/L at 20°C); its log Pow shows a significant dependence on the pH. No data on stability are provided.

Irritation, sensitisation
According to a study conducted in rabbits benzisothiazolinone (BIT) can be classified as a moderate irritant to skin. A study in rabbits classified the compound as a severe eye irritant.

A guinea pig maximization test classified BIT as a moderate contact sensitizer whilst the Buehler test classifies BIT as non-sensitising. Literature data for the local lymph node assay support a classification of BIT as a moderate dermal sensitizer (EC3 2.3%).

From a four week in-use study with humans only limited conclusions can be drawn due to the low number of subjects, no detailed description of pre-existing chloromethyl-isothiazolinone allergy and a significant variation in product use.

Several case reports in the dermatological literature describe allergic contact dermatitis to benzisothiazolinone, and some individuals wearing disposable powder-free polyvinyl chloride gloves containing 3 - 36 ppm benzisothiazolinone have developed allergic contact dermatitis (Aalto-Korte et al. 2007). According to the latter study, a concentration of 20 ppm benzisothiazolinone in a glove seems to be enough for sensitization. Moreover, in the context of occupational uses, benzisothiazolinone (BIT) is a well-documented contact allergen.

As has been seen with MCI/MI and now with MI itself, these isothiazolinones are important contact allergens for the consumer in Europe. Within the mixture, MCI is known to be the more potent allergen (EC3 0.009%). MI is less potent (EC3 1.9%) and is now permitted at up to 100 ppm in leave on and rinse off cosmetic products; contact allergy to MI itself is

now a considerable problem in Europe and this is of concern (Add. ref: 50-61). If BIT is to be used as a preservative in cosmetic products, it is essential that the level be sufficiently low to prevent a repeat of history.

It is recommended that the incidence of contact allergy to BIT and other isothiazolinones be monitored at regular intervals (e.g. annually), by reference to dermatology clinic data in Europe. Necessary early interventions can then be introduced to reduce exposures and hence contact allergy and allergic contact dermatitis as required.

Dermal absorption
An in vitro study with human skin has been provided. The mean value + 1SD can be used for calculating the MOS. This was 61.9% of the applied dose (1.29 µg/cm^2) when 0.01% benzisothiazolinone aqueous was applied.
The dermal absorption studies had not been performed with representative cosmetic formulations.

General toxicity
The acute toxicity of benzisothiazolinone upon oral or dermal administration to rats is low (LD50 of 1193 and 4115 mg/kg bw, respectively). The NOAEL derived from a subacute 28 day oral (gavage) toxicity study in rats was 12.63 mg a.i./kg bw/day. Subchronic toxicity was evaluated in a 90 day oral (gavage) study (according to OECD guideline 408) and provided a NOAEL of 8.42 mg/kg bw/day for the active ingredient. The NOAELs in the 28-day and 90-day studies were based on histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance and are therefore, not relevant for the safety assessment of benzisothiazolinone as a cosmetic ingredient.

Reproductive Toxicity
In a two generation study in rats with dietary administration benzisothiazolinone produced at 1000 ppm slight adult toxicity in the F1 generation in terms of lower body weight gain, and in both generations limiting ridge hyperplasia of the stomach together with incidences of squamous cell hyperplasia, forestomach gastritis, keratosis and erosion/ulcer. At this concentration, the growth of the offspring was slightly impaired and in the F2a offspring, there was a slight reduction in pup survival. At 500 ppm, there were incidences of limiting ridge hyperplasia in the stomach only. There were no adverse effects of treatment at 250 ppm, equivalent to an approximate overall mean intake of 24 mg/kg bw/day (active ingredient). The NOAEL was based on histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance and therefore, not relevant for the safety assessment of benzisothiazolinone as a cosmetic ingredient. Therefore, the NOAEL of 50 mg a.i./kg bw/day for the systemic effects of benzisothiazolinone will be used for the safety assessment.

Mutagenicity
Benzisothiazolinone has been tested for the induction of gene mutation in bacterial and mammalian cells treated *in vitro*, for clastogenicity on mammalian cells treated *in vitro*, for the induction of micronuclei in mice and for the induction of UDS in rats treated *in vivo*. The study on the induction of gene mutations on bacterial cells is inadequate due to the toxicity of the test item. The compound has been found to be clastogenic in mammalian cells treated *in vitro*. The compound has been found non mutagenic *in vitro*, non clastogenic and DNA damaging *in vivo*.

Carcinogenicity
No data provided

4. CONCLUSION

1. *Does SCCS consider benzisothiazolinone safe when used as a preservative up to a maximum authorised concentration of 0.01% in cosmetic products, based on the provided data?*

The SCCS considers benzisothiazolinone safe for use as a preservative in cosmetics products up to 0.01% with respect to systemic toxicity.

However, its sensitising potential is of concern.

2. *And/or does the SCCS have any scientific concern with regard to the use of benzisothiazolinone in cosmetic products?*

Sensitisation from related isothiazolinones is an important problem in consumers. This has occurred because there has been consumer exposure before safe levels of exposure relevant to sensitisation have been established. Benzisothiazolinone is a skin sensitiser in animal models with potency similar to methylisothiazolinone. Methylisothiazolinone, at 100 ppm (0.01%) in cosmetic products is causing contact allergy and allergic contact dermatitis in the consumer. Benzisothiazolinone is known to be a sensitiser in man and has induced sensitisation at *circa* 20 ppm in gloves.

There is no information on what may be safe levels of exposure to benzisothiazolinone in cosmetic products from the point of view of sensitisation.

Until safe levels of exposure have been established, the use of benzisothiazolinone in cosmetic products as a preservative or for other functions cannot be considered safe in relation to sensitisation.

5. MINORITY OPINION

Not applicable

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